Planta medica

Journal of Medicinal Plant Research

Editor - in - Chief
E. Reinhard, Univ. Tübingen
Pharmazeutisches Institut
Auf der Morgenstelle
7400 Tübingen

Editorial Board
H. P. T. Ammon, Tübingen
W. Barz, Münster
E. Reinhard, Tübingen
O. Sticher, Zürich
H. Wagner, München
M. H. Zenk, Bochum

Hippokrates Verlag
Stuttgart

Contens

Vol. 34/1978

© Printed in Germany

Index of Plant Names

Acalypha indica 410
Acronychia porteri 130
Aesculus indica 337
Aframomum melegueta 7
Alangiaceae 381
Alangium lamarckii 381
Aloe 113
Ammi majus 167
Anaphalis contorta 94
Andrachne colchica 408, 410
– *decaisnei* 409
Angelica archangelica 167
Apium graveolens 167
Apocynaceae 37, 390
Arnica longifolia 299
Asteraceae 299, 328
Atalantia roxburghiana 130

Baccharis crispa 328
Beyeria laeschenaultii 410
Bignoniaceae 219
Bridelia exaltata 408
– *monoica* 413
– *ovata* 408
– *tomentosa* 408
Bupleurum falcatum 160, 275, 294
Burkillanthus malaccensis 130

Cactaceae 207
Campanula rotundifolia 10
Capsicum spec. 7
Cassia angustifolia 430
– *laevigata* 319
Catharanthus 37, 259
Centranthus ruber 203
Cephaelis 381
Chrysanthemum parthenium 80
– *vulgare* 80
Cinchona ledgeriana 1, 16
– *succirubra* 18
Cinnamomum ceylanicum 294
Clausena excavata 130
Cnicus benedictus 80
Cnidoscolus stimulosus 410
– *texanus* 410
Colliguaja integerrima 410
Compositae 5, 79, 328
Convolvulus microphyllus 222
Crataegus oxyacantha 226
Crataeva religiosa 223
Crocus sativus 9
Cynara cannabinum 80
– *scolymus* 80

Digitalis lanata 44, 93, 225, 443
– *purpurea* 122
Dryopteris spec. 144, 397
– *carthusiana* 397
– *filix-mas* 398

Elateriospermum tapos 409
Ephedraceae 291
Ephedra spec. 291
Erythroxylum coca 14, 19
Escherichia coli 375
Eupatorium cannabinum 80
Euphorbiaceae 183, 408
Euphorbia boophthona 409
– *clutioides* 409
– *drummondii* 409
– *marginata* 183
– *peplus* 409
Euodia euneura 130
– *glabra* 130
– *macrocarpa* 130
– *roxburghiana* 130
Euonymus pendulus 211
Eurycoma longifolia 339

Fabaceae 414
Fusarium 232

Gentiana lutea 113, 114
– *pannonica* 114
– *pedicellata* 442
– *pneumonanthe* 176
– *punctata* 114
– *purpurea* 114
Glycosmis calcicola 130
Glycyrrhiza 294
Grossheimia macrocephala 80
Gymnanthes lucida 410
Gymnosporia montana 211

Hagenia abyssinica 144, 153
Harpagophytum procumbens 94

Hevea brasiliensis 409
– *spruceana* 410
Holarrhena floribunda 47

Indigofera suffructiosa 172
Inula helenium 80
Ipecacuanha (= Uragoga) 381, 430
Ipomoea muricata 93
Iris spec. 9

Jatropha angustidens 410
– *capensis* 410
Jurinea alata 80

Knightia deplanchei 66

Lathyrus hirsutus 420
– *odoratus* 420
– *silvestris* 420
– *tingitanus* 420
Lawsonia inermis 9
Leguminosae 172, 335
Liliaceae 216
Limonium gmelinii 218
– *perezii* 218
Lobelia 430
Loganiaceae 53, 57, 62, 264
Lygos raetum (= Genista r.) 335

Mallotus 144
Manihot aipa 409
– *carthaginensis* 409
– *glaziovii* 409
– *utilissima* 409
Marantaceae 323
Markhamia stipulata 219
Matricaria chamomilla 113
Menispermaceae 274
Mercurialis annua 410
Merendera caucasica 216
Merillia caloxylon 130
Merope angulata 130
Mesembryanthemum edule 226
Mitragyna parvifolia 259
– *speciosa* 26, 253
Momordica charantia 275
Myrtaceae 5

Nelia meyeri 226
Nicotiana glauca 73
– *tabacum* 73
Notocactus concinnus 207

Opuntia aurantiaca 207
– *brasiliensis* 207

Paeonia 294
Panax ginseng 294
Papaver bracteatum 135
– *orientale* 135
– *somniferum* 14, 135
Paramignya lobata 130
Petroselinum sativum 167
Pharnaceum corymbosum 409
– *suffruticosum* 409
Phyllanthus gasstroemii 409
– *lacunarius* 409
– *niruri* 409
– *speciosus* 409
Phytolacca americana 87
Pinellia ternata 294
Piper nigrum 7
Plumbaginaceae 218
Poranthera corymbosa 408
– *microphylla* 408
Pseudomonas aureofaciens 354
Psoralea corylifolia 168
Pycnarrhena manillensis 274
– *novoguineensis* 274

Ranunculus lanuginosus 10
Rauwolfia cumminsii 390
– *ivorensis* 390
– *liberiensis* 390
– *mombasiana* 395
– *vomitoria* 395
Rhamnus frangula 311
Rosaceae 153
Rubiaceae 26, 253, 381
Rutaceae 129, 338
Ruta graveolens 168

Scutellaria 294
Securinega ramiflora 409
– *suffruticosa* 408
Simarubaceae 339
Skimmia laureola 338
Smyrnium connatum 215
Staphylococcus aureus 346
Streptomyces antibioticus 363
– *arenae* 370
– *griseus* 346
– *violaceoruber* 372
Strychnos caespitosa 53
– *dale* 264
– *dolichothyrsa* 62

– *elaeocarpa* 264
– *gossweileri* 53
– *urceolata* 62
– *usambarensis* 57
– *variabilis* 57
Styrax officinalis 403
– *tonkinense* 9

Tetractomia roxburghii 130
– *tetrandra* 130
Thapsia villosa 218
Thaumatococcus daniellii 323
Tilia argentea 93
Tribulus terrestris 94, 188
Tricholepsis angustifolia 109
– *glaberrima* 109
– *procumbens* 109
Triglochin maritima 410
Trigonella foenum-graecum 414
Triphasia trifolia 130

Uragoga 381, 430

Valeriana mexicana 305
– *officinalis* 113, 120

Wigginsia arechavaletai 207

Zanthoxylum myriacanthum 130
Zizyphus 294
Zingiber officinale 7, 294
Zygophyllaceae 188

Subject Index

Acevaltrat 120
Actinorhodin 370
Acylglycosides 93
Aescin 337
Ajamalicinic acid 38
Ajmalan diacetate 392
Ajmalicine 27, 31, 37, 40, 254, 394
19-epi Ajmalicine 40
Ajmalicinine 394
Ajmaline 195, 394
Akagerine 264
Akuammigine 35, 254
Alangiside 381
Alatolide 80
Allergic contact dermatitis 299
Aloin 121
Aloinosid 121
Alstonine 40
Amarogentin 115
Amaropanin 115
Amaroswerin 115
Anethol 5
Anguidine 231
Anhydronium bases 390
Anthocyanins 323
Anthroneglycosides 311
Antibiotics 345
Antiphlogistic activity 97
Antitumor survey 129
Apigenin 226, 335
Arecolin 195
Aricine 394
Arnifolin 300
Atropin 195

Benzoic acid 9
Benzoisochromane quinones 373
Berbamine 274
Betainyl-anguidine 231
Bisabolol 116
Bisbenzylisoquinoline alkaloids 274
Bis Indolealkaloids 57, 62

Campesterol 215, 339
Capsaicin 6
Carvon 5
Corynoxine B 31
Cassia senna 311
Catharanthine 40
Cell suspension cultures 73
Cephaelin 195, 381
Cerotic acid 211
Chamazulen 116
Chavicine 6
Chinchonin 195
Chichonidin 195
Chinidin 195
Chinin 195
Chloroperoxidase 352
Chlorothricin 345, 362
Ciliaphylline 27, 31, 253
Cinnamic acid 222
Cinchonidine 14
Cinnamyl cinnamate 9
Cinnamylcocaine 14
Citronellol 426
Cnicin 80
Cocaine 14
Codeine 14, 195
Colchicin 195
Colchicine alkaloids 216
Concanavaline A 233, 423
Conessine 47, 49
Coniferyl benzoate 9
Coniin 195
Contact allergens 299
Convulsant 264
Corynantheal 394
Corynantheidine 26, 31, 254
Corynantheol 394
Corynoxeine 27, 31
Corynoxine 27, 31
Coumarins 338
p-Coumaryl- benzoate 9
Crocin 9
Cuscohygrine 14
Cupreine 14
Cyanogenic glucosides 408
Cyanogenesis 408
Cycloheximide 232
Cycloartenediol 109
Cynaropicrin 80
Cytosine arabinoside 232, 239

Daidzein 335
Demethylation 73
N-Demethylindolmycin 348

Deoxydiaboline 58
Desacetylretuline 58
Desmethoxycentaureidin 225
Diacetylajmaline 394
Dianthroneglycosides 311
Dia-Valtrate 203
Didrovaltrat 120, 306
Digoxin 44
Dihydroindoles 390
Dihydronorpurpeline 394
Dihydrophenylalanine 345, 357
Dimeric indole alkaloids 62
Dioscin 188
Diosgenin 188, 223, 414
DNA synthesis 231
Dolichantoside 53
Dulcitol 211

Emetin 195, 391, 430
Endolobine 394
En-In-Dicycloäther 116
Ephedrin 195, 291
Epipicraphylline 394
Epifriedelinol 211
Esdragol 5
Eugenol 5
Eupatoriopicrin 80

Fanchinoline 275
Flavones 176
Flavonoids 225, 319, 323, 328, 335
Flavonol glycoside 323
Frangularoside 311
Frenolicin 370
Friedelin 211
Furocoumarins 167
Furostanolbisglycoside 188

Geissospermine 40
Gentiopikrosid 115
Gingerols 6
Gitogenin 415
Glycoflavones 323
Granaticin 345, 369, 372
Griseusin 370
Grossheimin 80

Haemolysis 160
Harpagosid 97
HeLa cells 235
Helenalin 299
Helenalinacetate 299
Helenalinmethacrylate 299
Heteroyohimbine 390
Hevea brasiliensis 1
Hispidol 335
Hispidulin 444
Holadienine 47
Holarrheline 47
Holarrhesine 47
Homoacevaltrat 120
Homobaldrinal 305, 306
Homodidrovaltrat 120
Homovaltrat 120
Hydroxyurea 232
Hydroxykynurenic acid 218
Hygrine 14
Hypertensive activity 291

Immunosuppressiv activity 233
Indole alkaloids 37, 264, 390
Indolepyruvic acid 348
Indolmycenic acid 348
Indolmycin 345, 346, 348
Ionones 9
Ipecoside 381
Ipomine 93
Isocorynantheidine 253, 254
Isodidrovaltrat 120
Isofangchinoline 274
Isomitrafoline 26, 31
Isomitraphylline 26, 31, 254
Isoorientine 442
Isopayantheine 253, 254
Isoquinoline alkaloids 381
Isorhynchophylline 31, 254
Isoscoparine 176
Isospeciofoline 26, 31
Isostrychnobiline 57
Isotetrandrine 275
Isovaltrat 120, 306
Isovincoside 259
Isovitexine 176

Jaceosid 444
Javaphylline 254

Kaempferol 222
Kalafungin 370
Kribine 264

Lapachol 219
Lapachone 219

Laticifers 183
Lawson 9
Lectins 420
Leucocristinesulfat 40
Leucosidinesulfat 40
Leucosinesulfat 40
Lignan 403
Limacine 274
Linalool 5
Linamarin 409
Lobeline 430
Lochnerine 40
Louisfieserone 172
Luteolin 444
Lymphocytes 231

Manihotoxin 409
Maokonine 291
Marmesin 167
Matricin 116
Menthol 5
Methionine 346
β-Methylindolepyruvic acid 348
O-Methylpsychotrin 195
Mitraciliatine 31, 253, 254
Mitrafoline 26, 31
Mitragynine 26, 27, 31, 254
– oxindole B 31
Mitrajavine 254
Mitraphylline 26, 27, 31, 254
Monoterpenes 426
Morphin 14, 195
Mouse mastocytoma cells 233
Mucilages 207
Muscle-relaxant effects 264
Mycotoxic activity 232

Nanaomycin 370
Naphtocyclinone 345, 369, 370
Narcotin 195
Naringenin 335
Neogitogenin 415
Nepetrin 444
Nicotine 73, 195
Nordihydrotoxiferine 57
Normacusine 394
Normitoridine 394
Nornicotine 73
2-N-norobamegine 275
Norpurpeline 394
Norseredamine 394
Nortetraphyllicine 394

Obamegine 275
Ovalicin 231
Oxindole alkaloids 26, 259

Palmitone 219
Papaverin 195
Paradols 6
Parthenolide 80
Paulownin 219
Payantheine 26, 31, 254
Payanthine 26
Pectolinarigenin 225
Pentadecanolide 5
Pericyclivine 391, 394
Phaeantine 274
Phaseolunatin 409
Phloroglucinol derivatives 144, 153, 397
Phyllanthin 409
Phytohormones 73
Phytolaccosides 87
Pinitol 172
Piperetine 6
Piperine 6
Procyanidin 226
Protein synthesis 231
Protodioscin 188
Pseudoquaianolide 299
Psoralen 167
Psychotrin 195
Puromycin 232
Purpeline 394
Pycnamine 274
Pyrrolnitrin 345, 351

Quercetin 335
Quercetrin 337
Quinidine 14
Quinine 14
Quinoline 218

Radioimmunoassay 37
Repanduline 275
Rescinnamine 40, 394
Reserpine 40, 195, 394
Reticulocytes 233
Retuline 58
Rifampicin 233
Rhynchociline 27, 31, 253
Rhynchophylline 27, 31, 254

Rosmarinic acid 5
Rutaretin 167

Safranal 9
Saiko-Keishi-To 294
Saikosaponin 160, 275
Sandwicine 40
Sapogenins 414
Saponarine 176
Saponines 339
Sarpagan 390
Sarpagine 40, 394
Scopolamin 195
Sennoside 311, 430
Seredamine 394
Seredamine-17-O-trimethoxybenzoate 394
Serpentine 37, 40, 394
Serpentinic acid 38
Serum proteins 420
Sesquiterpene lactones 79, 299
Sesquiterpenes 231
Shogaols 6
Siaresinolic acid 9
Sitosterol 172, 211, 215, 219, 222, 223, 335, 337, 339
Specifoline 31
Speciociliatine 26, 31, 254
Speciofoline 27
Speciogynine 26, 31, 254
Specionoxeine 26
Speciophylline 26, 31
Spectinomycin 345, 360
Spleen lymphocytes 233
Steroidal alkaloids 47
Sterols 339
Stigmasterol 215, 339
Strictosidine 259
Strychnobiline 57
Styraxin 403
Tanacetin 80
Taxiphyllin 409
Tectol 219
Testicular function 275
Tetrahydroalstonine 35, 40
Tetrandine 275
Tetraphyllicine 394
Thebain 195
Thymol 5
Tiliroside 93
Tribuloside 93, 94
Trichophyton 351
Tricontane 223
Tricontanol 223
Triglochinin 408, 410
Triterpenoidsaponins 87
Tropacocaine 14
Tropane alkaloids 66
Tropanol 195
Tryptophan 346

Vaccinia virus 233
Valepotriate 305
Valepotriates 203
Valtrat 120, 306
Valtrathydrine 120
Vanilline 9
Verrucarin 231
Vinblastine 195
Vinblastinesulfat 40
Vincamin 40, 195
Vincoside 259
Vincristine 195
Vindoline 40
Vindolinine 40
Vomalidine 394

Yohimbine 40, 195, 390, 394

Contents

ABE, H., M. SAKAGUCHI, H. KONISHI, T. TANI, S. ARICHI, The Effects of Saikosaponins on Biological Membranes. I. The Relationship between the Structures of Saikosaponins and Haemolytic Activity. 160

ABE, H., S. ODASHIMA, S. ARICHI, The Effects of Saikosaponins on Biological Membranes. II. Changes in Electron Spin Resonance Spectra from Spin Labelled Erythrocyte and Erytrocyte Ghost Membranes 287

ADESINA, S. K., J. B. HARBORNE, The Occurrence and Identification of Flavonoids in *Thaumatococcus danielli* BENTH 323

ARENS, H., J. STÖCKIGT, E. E. WEILER, M. H. ZENK, Radioimmunoassays for the Determination of the Indole Alkaloids Ajmalicine and Serpentine in Plants 37

ÄYRÄS, P. and C.-J. WIDÉN, NMR Spectroscopy of Naturally Occuring Phloroglucinol Derivatives. Part I: The Carbon-13 Spectral Properties of Some Model Compounds 144

BANDONI, A. L., J. E. MEDINA, R. V. D. RONDINA, J. D. COUSSIO, Genus *Baccharis* L., I: Phytochemical Analysis of a non Polar Fraction from *B. crispa* SPRENGEL 328

BARE, C. E., V. K. TOOLE, W. A. GENTNER, Temperature and Light Effects on Germination of *Papaver bracteatum* LINDL., *P. orientale* L., and *P. somniferum* L. 135

BARZ, W., M. KETTNER, W. HÜSEMANN, On the Degradation of Nicotine in *Nicotiana* Cell Suspension Cultures 73

BHATIA, R. K., D. R. LOHAR, D. D. CHAWAN, S. P. GARG, Chemical Control of Yield of Sennosides in *Senna* Leaves 437

BISHT, N. P. S. and R. SINGH, Chemical Studies of *Convolvulus microphyllus* SIEB. 222

BŁOSZYK, E., B. GEPPERT, B. DROŻDŻ, Quantitative Determination of Sesquiterpene Lactones in Plant Material by Infrared Spectroscopy 79

BOWEN, IAN, H. and JOHN R. LEWIS, Rutaceous Constituents, Part 10: A Phytochemical and Antitumor Survey of Malayan Rutaceous Plants 129

BURRET, E., A. J. CHULIA, A. M. DEBELMAS, K. HOSTETTMANN, Présence d'Isoscoparine, d'Isoscoparine -7-O- Glucoside et de Saponarine dans *Gentiana pneumonathe* L. (Presence of Isoscoparine, Isoscoparine -7-O- Glucoside and Saponarine in *Gentiana pneumonanthe* L.) 176

CHARI, U. M., M. JORDAN, H. WAGNER, Structure Elucidation and Synthesis of Naturally Occuring Acylglycosides; Structure of Tiliroside, Tribuloside and Ipomine 93

CHAWLA, A. S., V. K. KAPOOR, P. K. SANGAL, Cycloart – 23 ene – 3β, 25 – diol from *Tricholepsis glaberrima* 109

CHULIA, A. J., K. HOSTETTMANN, M. L. BOUILLANT, A. M. MARIOTTE, Contribution à l'Etude du Genre *Gentiana*. Un 3'-O-Glucoside d'Isoorientin chez *Gentiana pedicellata* (Contribution to the Study of the Genus *Gentiana*. A 3'-O-Glucoside of Isooriendine in *Gentiana pedicellata)* 442

COUNE, C., L. ANGENOT, Le Dolichantoside, un Alcaloide Nouveau du *Strychnos gossweileri* EXCELL (Dolychantoside, a New Alkaloid from *Strychnos gossweileri*) 53

DALL'OLIO, G., B. TOSI, A. BRUNI, A New Rapid Fluorescent Labelling Method for the Detection of the Latex Tissues in Euphorbiaceae Plants 183

DIXIT, V. P., P. KHANNA, S. K. BHARGAVA, Effects of *Momordica charantia* L. Fruit Extract on the Testicular Function of Dog 280

DOMÍNQUEZ, X. A., C. MARTÍNEZ, A. CALERO, X. A. DOMÍNQUEZ jr., M. HINOJOSA, A. ZAMUDIO, W. WATSON, V. ZABEL, Mexican Medicinal Plants, XXXI: Chemical Components from „Jiquelite", *Indigofera suffruticosa*, MILL. 172

ELSAYED, M.A., ABDEL SALAM, M. A., ABDEL SALAM, N. A., MOHAMMED, Y. A., Spectrophotometric Determination of Emetine and Lobeline by Charge Transfer Complexation 430

El Sherbaini, A. E. A., H. I. El Sissi, M. A. M. Nawwar, M. A. El Ansari, The Flavonoids of the Seeds of *Lygos raetum* 335

Erdös, A., R. Fontaine, H. Friehe, R. Durand, Th. Pöppinghaus, Beitrag zur Pharmakologie und Toxikologie verschiedener Extrakte, sowie des Harpagosids aus *Harpagophytum procumbens* D. C. (Contributions to the Pharmacology and Toxicology of different Extracts as well as the Harpagosid from *Harpagophytum procumbens*) 97

Floss, H. G., C.-J. Chang, O. Mascaretti, K. Shimada, Studies on the Biosynthesis of Antibiotics 345

Franke, W., B. Brümmer, Flüchtige Stoffwechselprodukte aus *Ascoidea hylecoeti* (Volatile Substances from *Ascoidea hylecoeti*) 332

Franke, W. und B. Brümmer, Terpene aus *Ascoidea hylecoeti* (Terpenes from *Ascoidea hylecoeti*) 426

Handjieva, N. and v. G. Zaikin, DIA-Valtrate a New Valepotriate from *Centranthus ruber* (L.) DC. 203

Hardmann, R. and R. G. Stevens, The Influence of NAA and 2,4–D on the Steroidal Fractions of *Trigonella foenum-graecum* Static Cultures 414

Hartmann, G. R., H. Richter, E. M. Weiner, W. Zimmermann, On the Mechanism of Action of the Cytostatic Drug Anguidine and of the Immunosuppressive Agent Ovalicin, two Sesquiterpenes from Fungi 231

Hegnauer, R., Arzneipflanzen, Gestern, Heute und Morgen (Medicinal plants, in the past, today and tomorrow) 1

Herrmann, H. D., G. Willuhn, B. M. Hausen, Helenalinmethacrylate, an new Pseudoguaianolide from the Flowers of *Arnica montana* L. and the Sensitizing Capacity of their Sesquiterpene Lactones 299

Hiermann, A. und Th. Kartnig, Flavonoide in den Blättern von *Digitalis lanata* 2. Mitteilung (Flavonoids in the Leaves of *Digitalis lanata*, 2nd Communication) 225

Hiermann, A., Flavonoide in den Blättern von *Digitalis lanata*, III. (Flavonoids in the Leaves of *Digitalis lanata*, III) 443

Hoyer, G. A., A. Huth, J. Nitschke, Ch. v. Szczepanski, Holarrhesin, ein neues Steroidalkaloid aus *Holarrhena floribunda* (Holarrhesin, a new Steroidal Alkaloid from *Holarrhena floribunda*) 47

Huhtikangas, A., A. Huurre, M. Lounasmaa, Studies on the Biosynthetic Origin of the Acyl Side Chains of *Dryopteris* Fern Constituents 397

Ikram, M., M. Israr Khan, N. Kawano, Chemical Investigation of *Aesculus indica* 337

Innocenti, G., F. Dall'Acqua, P. Rodighiero, G. Caporale, Biosynthesis of O-Alkylfurocoumarins in *Angelica archangelica* 167

Iwu, M. M. and W. E. Court, The Alkaloids of *Rauwolfia cumminsii* 390

Joshi, K. C., R. K. Bansal and R. Patni, Chemical Constituents of *Gymnosporia montana* and *Euonymus pendulus* 211

Joshi, K. C., P. Singh, R. T. Pardasani, Chemical Constituents of the Stem Heartwood of *Markhamia stipulata* 219

Kolodziej, H. und H. Friedrich, Konstitutionsermittlung eines Procyanidins in *Nelia meyeri* 226

Lemli, J., J. Cuveele, Les transformations des hétérosides anthroniques pendant le séchage des feuilles de *Cassia senna* et de *Rhamnus frangula*, XXXI (Transformation of anthronglycosides by drying of the leaves of *Cassia senna* and *Rhamnus frangula*. Investigations on anthraquinone drugs, XXXI) 311

Lounasma, M., G. Massiot, ^{1}H Nuclear Magnetic Resonance Spectral Analysis of Some *Knightia deplanchei* Alkaloids 66

Lounasmaa, M. et P. Varenne, Dérivés Phloroglucinoliques d'*Hagenia abyssinica*. V: Spectrometrie de Masse en Ionisation Chimique de la Kosotoxine, de la Protokosine et de l'α-Kosine. (Phloroglucinol Derivatives of *Hagenia abyssinica*. V: Chemical Ionisation Mass Spectrometry of Kosotoxin, Protokosin and α-Kosin) 153

Lounasmaa, M. and H. Puhakka, Stereoselective Reduction of 1-Acetyl-3, 4, 6, 7, 12, 12b – hexahydroindole (2,3- a) quinolizine to the Corresponding 1 – (1-Hydroxyethyl) Derivative 180
Méndez, J., 6-Hydroxykynurenic Acid from Leaves of *Limonium* Species 218
Moyna, P. and J. D. Di Fabio, Composition of Cactaceae Mucilages 207
Nakagura, N., G. Höfle, D. Coggiola, M. Zenk, The Biosynthesis of the Ipecac Alkaloids and of Ipecoside and Alangiside 381
Oei-Koch, A. Lj. Kraus, Inhaltsstoffe von *Eurycoma longifolia,* I: Sterole, Saponine (Constituents of *Eurycoma longifolia* I: Sterols, Saponines) 339
Père, D., P. Rougé, S. Lascombes, Precipitation des Glycoproteines du Serum Humain Normale par les Lectines de quelques Especes de *Lathyrus* (Precipitation of Human Serum Glycoproteins by the Lectins from Various *Lathyrus* Species) 420
Rolfsen, W., L. Bohlin, S. K. Yeboah, M. Geevaratne, R. Verpoorte, New Indole Alkaloids of *Strychnos dale* and *Strychnos elaeocarpa* 264
Sethi, V. K., M. P. Jain and R. S. Thakur, Chemical Constituents of *Crataeva religiosa* 223
Shellard, E. J., P. J. Houghton, Masechaba Resha, The *Mitragyna* Species of Asia, Part XXXI: The Alkaloids of *Mitragyna speciosa* Korth from Thailand 26
Shellard, E. J., P. J. Houghton, Masechaba Resha. The *Mitragyna* Species of Asia, Part XXXII The Distribution of Alkaloids in Young Plants of *Mitragyna speciosa* Korth grown from Seed obtained from Thailand 253
Sood, S., B. O. Gupta, S. K. Banerjee, Constituents of *Skimmia laureola* 338
Stahl, E. und E. Willing, Extraktion von Alkaloiden mit überkritischen Gasen in direkter Koppelung mit der Dünnschicht-Chromatographie (Extraction of Alkaloids with Supercritical Gases in Direct Coupling with Thin – Layer Chromatography) 192
Sugaya, A., T. Tsuda, E. Sugaya, M. Takato, K. Takamura, Effect of Chinese Medicine „Saiko-Keishi-To" on the Abnormal Bursting Activity of Snail Neurons 294
Tamada, M., K. Endo, H. Hikino, Maokinine, Hypertensive Principle of *Ephedra* Roots 291
Tits, M. J. G., L. Angenot, Nouveau Alcaloides bis – Indoliques dans L'Ecorce des Racines du *Strychnos variabilis* (New Bis – Indole Alkaloids in the Bark of *Strychnos variabilis* Root) 57
Tittel, G., V. M. Chari, H. Wagner, HPLC-Analyse von *Valeriana mexicana* Extrakten (HPLC-Analysis of *Valeriana mexicana* Extracts) 305
Tiwari, R. D., J. Sing, Phytochemical Investigation of *Cassia laevigata* Pods, Part I: Isolation and Structural Studies of two new Flavonol Glycosides 319
Tomowa, M. P. und R. Gjulemetowa, Steroidsaponine und Steroidsapogenine. VI: Furostanolbisglykosid aus *Tribulus terrestris* L. (Steroidsaponines and Steroidsapogenines. VI: Furostanolbisglycoside from *Tribulus terrestris* L.) 188
Ulubelen, A. and A. Ates, Steroidal Compounds of *Smyrnium connatum* 215
Ulubelen, A. and M. Tanker, Alkaloids of *Merendera caucasica* 216
Ulubelen, A., Y. Saiki, H. Lotter, V. M. Chari, H. Wagner, Chemical Components of *Styrax officinalis* IV. The Structure of a New Lignan Styraxin 403
van Valen, F.: Contribution to the Knowledge of Cyanogenesis in Angiosperms 408
Verpoorte, R., E. W. Kodde, A. Baerheim-Svendsen, A Chromatographic Comparison of *Strychnos dolichothyrsa* and *Strychnos urceolata* 62
Verpoorte, R., A. H. M. van Rijzen, J. Siwon, A. Baerheim-Svendsen, Tertiary alkaloids of *Pycnarrhena novoguineensis.* Studies on Indonesian Medicinal Plants I 274
Wichtl, M., Drogenanalytik und Arzneibuch, kritisch betrachtet (Drug Analysis and Pharmakopoea, a Critical Survey) 113
Woo, W. S., S. S. Kang, H. Wagner, O. Seligmann, V. M. Chari, Triterpenoid Saponins from the Roots of *Phytolacca americana* 87

Planta medica
Journal of Medicinal Plant Research

Editor - in - Chief
E. Reinhard, Univ. Tübingen
Pharmazeutisches Institut
Auf der Morgenstelle
7400 Tübingen

Editorial Board
H. P. T. Ammon, Tübingen
W. Barz, Münster
E. Reinhard, Tübingen
O. Sticher, Zürich
H. Wagner, München
M. H. Zenk, Bochum

Hippokrates Verlag
Stuttgart

November 1978
Vol. 34
No. 3

Review Article

On the Mechanism of Action of the Cytostatic Drug Anguidine and of the Immunosuppressive Agent Ovalicin, two Sesquiterpenes from Fungi[1]

Guido R. Hartmann, Hartmut Richter, Erika M. Weiner and Wolfgang Zimmermann

Institut für Biochemie der Ludwig-Maximilians-Universität München, Bundesrepublik Deutschland.

Key Word Index: Anguidine; Verrucarin; Ovalicin; Fungal Sesquiterpenes; Lymphocytes; DNA Synthesis, Protein Synthesis.

Abstract

The sesquiterpene betainyl-anguidine and the structurally related verrucarin A at the low concentration of 10^{-8} mol/l inhibit protein synthesis in lymphocytes from mouse spleen rapidly after addition to the cell culture. DNA synthesis is blocked at similarly low concentrations whereas RNA synthesis is much

[1] Dedicated to Professor ADOLF BUTENANDT on the occasion of his 75th birthday.

less reduced. The following observations support the notion that DNA synthesis is blocked via inhibition of protein synthesis: (i) DNA synthesis in a cell-free system is not reduced by the drug; (ii) in cell culture protein synthesis is inhibited more rapidly and at lower concentrations than DNA synthesis; (iii) inhibitors of protein synthesis such as cycloheximide or puromycin are similar to betainyl-anguidine in their action on lymphocytes whereas specific inhibitors of DNA synthesis such as cytosine arabinoside or hydroxyurea only partially reduce protein synthesis in lymphocytes.

The sesquiterpene ovalicin at a concentration of 10^{-10} mol/l acts as a very potent inhibitor of DNA synthesis in proliferating lymphocytes and in lymphoma cells. RNA and protein synthesis are only weakly affected. The following observations support the conclusion that DNA synthesis is blocked only indirectly: (i) DNA synthesis in a cell-free system is not reduced by ovalicin; (ii) a cell-free system for DNA synthesis prepared from ovalicin-treated lymphocytes shows an impaired synthetic activity; (iii) in cell culture the action of ovalicin is not immediate and requires a 8–15 hour period of incubation.

Introduction

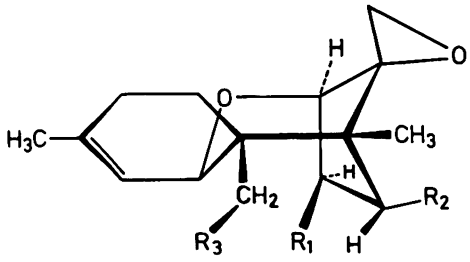
Fungi produce a large number of chemical compounds which are highly toxic either for bacteria or for animals and plants. They are toxic because they inhibit biochemical reactions essential for sustaining life. Their unusual specificity for either animals and plants or bacteria is based on fundamental differences existing between molecular constituents of the prokaryotic and eukaryotic cell. If the receptor for the target of such a toxic compound occurs only in a eukaryotic cell exclusively this type of cell will be affected. The specificity of action is particularly pronounced if such a compound possesses a very high affinity for its target. Then very small concentrations are sufficient for the inhibitory action to be observed. If higher concentrations (10^{-6} – 10^{-3} mol/l) are required the high specificity of action may be lost because weak forces such as hydrophobic interactions may provoke unspecific binding of these compounds also to other cellular constituents with concomitant impairment of their biological functions. Therefore compounds which elicit biological effects at very low concentration command higher interest.

In recent years the effort to suppress the uncontrolled growth of tumor cells and other rapidly proliferating cells such as committed lymphocytes has stimulated a general interest in compounds which act cytostatic for eukaryotic cells.

In this report the mode of action of two different compounds will be discussed which exhibit a high cytostatic activity at very low concentration and act exclusively against eukaryotic cells. Chemically both compounds are distantly related since they possess the basic structure of a sesquiterpene.

I. Anguidine and related compounds

Anguidine is produced by various species of *Fusaria* and shows mycotoxic



anguidine:
trichothec-9-ene-3,4,15-triol,12,13-epoxy, 4,15-diacetate, (3α, 4β):

$R_1 = OH$; $R_2 = R_3 = O\text{-}\overset{\overset{O}{\|}}{C}\text{-}CH_3$

betainyl-anguidine (chloride):

$R_1 = O\text{-}\overset{\overset{O}{\|}}{C}\text{-}CH_2\text{-}\overset{+}{N}(CH_3)_3\ Cl^-$; $R_2 = R_3 = O\text{-}\overset{\overset{O}{\|}}{C}\text{-}CH_3$

verrucarin A:

$R_1 = H$; R_2, R_3 = dicarbonic acid ester of:

$$\text{-O-}\overset{\overset{O}{\|}}{C}\text{-}\overset{\overset{OH}{|}}{CH}\text{-}\overset{\overset{CH_3}{|}}{CH}\text{-}CH_2\text{-}CH_2\text{-O-}\overset{\overset{O}{\|}}{C}\text{-CH=CH-CH=CH-}\overset{\overset{O}{\|}}{C}\text{-O-}$$

activity. Chemically it is closely related with the group of verrucarins [1]. Cytostatic activity of anguidine is observed at a concentration of 0.5×10^{-8} mol/l in mouse mastocytoma cells as well as in human tumor cells (KB cells). At a similarly low concentration the propagation of the DNA containing vaccinia virus and its cyptopathogenic effect is suppressed [2]. With respect to the molecular target of anguidine it has been observed previously that protein synthesis in a cell culture is inhibited at a concentration of 3×10^{-7} mol/l in eukaryotic cells such as rabbit reticulocytes or mouse mastocytoma cells. At the same concentration, however, DNA synthesis is equally blocked [2]. Synthesis of proteins and of DNA is achieved by biochemically very different processes. Therefore the question arises if anguidine acts on such unlike biosynthetic pathways by two different independent modes of action. This ist not a far-fetched question. For example, it has been observed that the antibiotic rifampicin specifically blocks RNA synthesis in bacteria [3]. On the other hand it inhibits the multiplication of vaccinia virus in cell cultures by a completely different mechanism [4]. The simultaneous inhibition of two different biochemical reactions, however, may also be explained without the assumption of two different modes of action if both reactions are tightly coupled *in vivo*.

In view of the observed possibly immunosuppressive activity [2] we have studied the molecular mode of action of anguidine in spleen lymphocytes from mice. In the absence of an antigenic or mitogenic stimulus lymphocytes are metabolically very inactive. Lymphocytes may be induced to proliferate by mitogens such as concanavalin A as is indicated by a strongly increased synthesis of RNA and proteins followed by a rapid synthesis of DNA after 25 h of incubation. In most of our experiments we have used the chemically modified derivative betainyl-anguidine which exhibits the same biological effects as anguidine but is much better soluble in water. If betainyl-anguidine is added simultaneously with mitogen and radioactively labelled leucine to lymphocytes in cell culture the incorporation of the radioactively labelled amino acid into proteins, determined after a 20 h incubation period, is reduced by more than the half in the presence of only 1×10^{-8} mol/l inhibitor.

Table I

Inhibition of protein synthesis in lymphocytes by betainyl-anguidine or verrucarin A.
2 μg concanavalin A, 10 nmol mercaptoethanol, 5 μCi (^{3}H)leucine (resulting specific radioactivity in the medium 13 Ci/mol) and inhibitor (as indicated) were added to 10^6 B6D2 mouse lymphocytes from spleen (preincubated at 37° C for 10–20 h) in Eagle's MEM containing 5 % fetal calf serum (38) (total volume 1.0 ml). After incubation for 20 h at 37° C incorporation of radioactive label into acid insoluble material was determined (39) (control in the absence of betainyl-anguidine: 4731 ± 149 counts × min^{-1}; control in the absence of verrucarin A: 8531 ± 914 counts × min^{-1}).

inhibitor	concentration (M)	inhibition of protein synthesis in cell culture (%)
betainylanguidine	4×10^{-9}	20
	1×10^{-8}	79
verrucarin A	4×10^{-9}	30
	1×10^{-8}	83

Table II

Influence of the cell cycle on the inhibitory action of verrucarin A.
The experiments were performed essentially as described in table I except that 2×10^{-8} mol/l verrucarin A and (^{3}H)leucine were added to the cultures at the time indicated after addition of concanavalin A.

verrucarin A added at (h)	pulse of [3H]leucine (h)	incorporation (cpm)	incorporation in absence of drug (cpm)
7.	8 – 10	69	412
15.	16 – 18	81	1079
39.	40 – 42	45	447

The same effect is observed with verrucarin A, a compound closely related to anguidine (table I). Cellular protein synthesis is blocked rather rapidly. Incubation for only 3 h with 2×10^{-8} mol/l verrucarin A is sufficient for a very strong inhibitory effect to be observed. This is observed no matter whether toxin is added 7, 15 on 39 hours after the mitogen (table II). To determine whether the synthesis of all cellular proteins is reduced to the same extent the following experiment was performed: 2×10^{-8} mol/l inhibitor was added to stimulated lymphocytes which was followed by radioactively labelled methionine one hour later. After two more hours the cellular proteins were separated by polyacrylamide gel electrophoresis in presence of dodecylsulfate. Except for a single protein zone migrating with the relative electrophoretic mobility of 0.52 the synthesis of all other radioactively labelled proteins is uniformly reduced (Fig 1). This is also true for the biosynthesis of histones as will be discussed

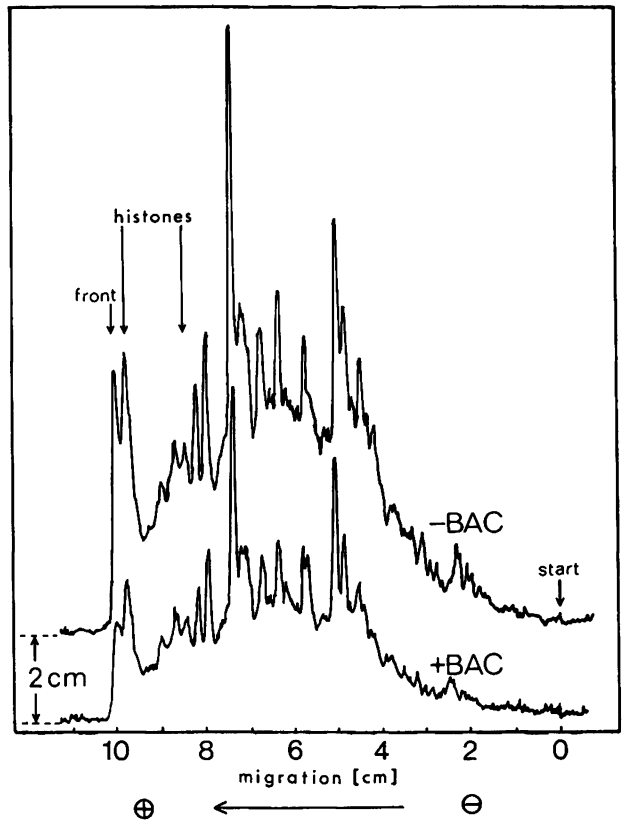


Fig. 1. Gel-electrophoretic analysis of radioactive proteins synthesized during incubation of stimulated lymphocytes with (^{35}S) methionine in the presence or absence of betainyl-anguidine. 5×10^7 lymphocytes stimulated as described in table III, were incubated for 39 h at 37° C. 1 h after addition of 2×10^{-8} M betainyl-anguidine (BAC) 0.25 mCi (^{35}S) methionine (specific radioactivity 20 Ci/mol) were added and the incubation continued for 2 h. Subsequently the cells from 4.5 ml of the incubation mixture were isolated by centrifugation. After washing with cold 0.85 % NaCl half of the cells were lysed for 10 min at room temperature by suspending them in about two volumes 60 mM tris-HCl pH 6.8 containing 10 % glycerol and 2.7 % dodecylsulfate (total volume 50 μl). 20 μl of this mixture (equivalent to 10^6 cells) were applied to a slab gel containing a linear gradient of 5–15 % acrylamide with 0.1 % dodecylsulfate. Electrophoresis was carried out at 60 V overnight [43]. Autoradiography was performed with the dried slab gel for 6 days. The developed film was scanned with a densitometer.

below. Histones are proteins tightly associated with DNA in eukaryotic cells.

These observations support the conclusion that in lymphocytes anguidine and related compounds act in general at very low concentration on protein synthesis. Furthermore, the action of anguidine and its derivatives is not restricted to a certain type of cell such as lymphocytes or to a certain species of animal as follows from experiments with rabbit reticulocytes [5] and HeLa cells [6]. Since they are effective at

lower concentrations than other inhibitors of protein synthesis such as puromycin or cycloheximide (table VI and VIII and unpublished experiments) their general application in biochemical and biological experiments is suggested.

On the other hand, derivatives of anguidine also act as very potent inhibitors of DNA synthesis as is shown by the following experiment: inhibitor is added together with mitogen to a cell culture of stimulated lymphocytes. When DNA synthesis is determined by incubation with radioactively labelled thymidine a 50% inhibition is observed in the presence of only 0.7×10^{-8} mol/l of the drug, similar to the inhibition of protein synthesis. Doubling of the concentration of the inhibitor leads to a complete inhibition. Verrucarin A is even more active. DNA synthesis is inhibited at a 10 fold lower concentration than protein synthesis (table III). Obviously anguidine is a very potent

Table III

Inhibition of DNA synthesis in lymphocytes by betainyl-anguidine, verrucarin A or ovalicin studied in cell culture and in a cell-free system.

100 μg concanavalin A, 0.5 μmol mercaptoethanol and betainyl-anguidine were added to 5×10^7 lymphocytes in medium (see table I) (total volume 45 ml). After 38 h incubation at 37° C 0.9 ml aliquots of the cell suspension were transferred to test tubes containing 1 μCi (^{3}H)thymidine (specific radioactivity 6.7 Ci/mmol). Incorporation of radioactive label into acid insoluble material was determined after 8 h incubation at 37° C. Control without betainyl-anguidine: 26817 ± 1351 counts $\times$ min^{-1}. The experiments with verrucarin A in cell culture were performed essentially as described in table I except that (^{3}H)leucine was omitted. After 36 h incubation 0.1 ml medium containing 1 μCi (^{3}H) thymidine (specific radioactivity 2 Ci/mmol) and 10 nmol mercaptoethanol was added. 18 h later incubation was terminated. Control wihout verrucarin A: 9016 ± 1784 counts $\times$ min^{-1}. Experiments with ovalicin in cell culture: 1 μg concanavalin A and inhibitor was added to 10^6 lymphocytes preincubated for 12 h. After 38 h 1 μCi (^{3}H)thymidine (specific radioactivity 6.7 Ci/mmol) was added and incubation continued for 3 h. Control without ovalicin: 61843 ± 2313 counts $\times$ min^{-1}. Synthesis of DNA in a cell-free system was measured essentially as described [7]. Nuclei were from lymphocytes stimulated for 46 h with concanavalin A in presence of 10 μM mercaptoethanol in the experiment with betainyl-anguidine (as in table I). In the experiment with ovalicin mercaptoethanol was left out. Incubation of the cell-free system was 45 min at 37° C. Control without betainylanguidine: 1890 counts $\times$ min^{-1} $\times$ 10^{-6} nuclei; control without ovalicin: 436 counts $\times$ min^{-1} $\times$ 10^{-6} nuclei.

inhibitor	concentration of drug (M)	inhibition of DNA synthesis (%) in cell culture	in a cell-free system
betainyl-anguidine	4×10^{-9}	12	–
	1×10^{-8}	98	–
	2×10^{-6}	–	7
verrucarin A	4×10^{-9}	33	–
	1×10^{-9}	96	–
ovalicin	2×10^{-10}	50	–
	4×10^{-10}	73	–
	3×10^{-6}	75	0

inhibitor of the synthesis of proteins as well as of DNA in lymphocytes. This fact has to be taken into consideration when discussing the molecular mode of action of this drug. In the case of verrucarin A DNA synthesis is even more sensitive than protein synthesis. Consequently we have to investigate the question if anguidine derivatives are double-headed inhibitors or if they block a process common to both protein and DNA synthesis.

Such a process would be the production of energy in the form of ATP required for both biosynthetic reactions. However this is not the case as is shown by the following argument. Biosynthesis of RNA also requires energy in the form of ATP. However, RNA synthesis in lymphocytes is much less reduced by a concentration of the inhibitor which inhibits protein synthesis distinctly within 3 h. Even 100 fold higher concentrations of the drug reduce RNA synthesis by only 50% (table IV). Such a relatively weak effect on RNA synthesis has been previously observed in mouse mastocytoma cells [2]. Of course, there are many other biochemical pathways which precede both DNA and protein synthesis. Any of them could be the target of anguidine. Instead of discussing them separately we would like to mention several arguments which support the notion that anguidine and its derivatives block DNA synthesis via the inhibition of protein synthesis.

(i) DNA synthesis in a cell-free system ist not inhibited by derivatives of anguidine. This process can be studied with nuclei obtained from stimulated lymphocytes [7]. During incubation of this system the activated precursors of DNA, the four deoxynucleoside

Table IV

Comparison of inhibitory action of betainyl-anguidine on RNA- and protein synthesis. Inhibition of protein synthesis (3 h incubation): similar to experiments described in Fig 1 except that after 40 h incubation 0.9 ml aliquots of the cell suspension was added to 0.1 ml Hank's solution containing inhibitor. After 1 h (^{3}H)leucine was added and incorporation determined after another 2 h at 37° C (control without drug: 16229 ± 3010 counts × min^{-1}); 20 h incubation: essentially as described in table I (control without drug 4731 ± 149 counts × min^{-1}). Inhibition of RNA synthesis: 3 h incubation with drug: drug was added to 10^6 lymphocytes (table 1), 18 h after concanavalin A. 2 hours later 50 nCi (^{14}C)uridine (specific radioactivity 415 (Ci/mol) was added and incorporation into acid insoluble material determined after 1 h (control without drug 693 ± 21 counts × min^{-1}). 12 h incubation with drug: concanavalin A, (^{14}C)uridine and drug were added together to the lymphocytes. Incorporation was determined after 12 h incubation (control without drug: 5094 ± 409 counts × min^{-1}).

betainyl-anguidine (M)	percent inhibition of incorporation of leucine after		uridine after	
	3 h	20 h	3 h	12 h
	incubation with drug		incubation with drug	
2×10^{-8}	62	95	28	41
1×10^{-7}	96	100	–	50
2×10^{-6}	–	–	52	69

Table V

Dependence of inhibitory action of betainyl-anguidine on protein or DNA synthesis on the time of incubation.

Stimulated lymphocytes as described for the experiments in cell culture with betainyl-anguidine in table III were used. Short incubation with drug: 40–43 h after addition of concanavalin A; long incubation with drug: 0–40 h after addition of concanavalin A; protein synthesis was measured by incubation with (^{3}H)leucine for 2 h; DNA synthesis was determined by incubation with (^{3}H)thymidine for 2 h.

incubation period with betainyl-anguidine	molar concentration of drug required for 50 percent inhibition of	
	protein synthesis	DNA synthesis
short	$1{,}5 \times 10^{-8}$	$5{,}0 \times 10^{-8}$
long	$0{,}5 \times 10^{-8}$	$0{,}5 \times 10^{-8}$

triphosphates dATP, dTTP, dGTP and dCTP, are incorporated into DNA Even in the presence of 2×10^{-6} mol/l betainylanguidine this reaction is only slightly reduced (table III).

(ii) In a cell culture protein synthesis is inhibited more rapidly and at lower concentration of the drug than DNA synthesis. Comparing the inhibitory effects of betainyl-anguidine observed after a long or a short incubation period it is immediately obvious that the inhibition of protein synthesis decreases much less than that of DNA synthesis upon shortening the incubation time. The concentration of the inhibitor has to be increased by a factor of ten to inhibit DNA synthesis to the same extent as protein synthesis during a short exposure to the drug (table V).

(iii) Specific inhibitors of protein synthesis such as puromycin or cycloheximide inhibit DNA synthesis in lymphocytes similarly to the derivatives of anguidine. This conclusion is derived from the observation that puromycin or cycloheximide inhibit DNA synthesis in stimulated lymphocytes at the same concentration as protein synthesis. Similar to betainyl-anguidine the effect of cycloheximide on DNA synthesis increases with the length of the exposure time. Smaller concentrations of the inhibitor are sufficient at a long incubation time (table VI).

All these observations are consistent with the hypothesis that protein biosynthesis is the primary target of anguidine and its derivatives. Subsequently DNA synthesis ceases. This effect is due to the tight coupling of DNA synthesis to protein synthesis in eukaryotic cells [8] which has been demonstrated with a large number of inhibitors of protein synthesis and by use of amino acid analogues in many different types of cells [9–15]. Probably the tight coupling is mediated by the process of histone biosynthesis which is absolutely required for the formation of chromatin [16–18]. DNA biosynthesis can not be the primary target of anguidine. Otherwise any blocking of DNA synthesis by specific inhibitors should lead to a general termination of protein biosynthesis. This question was studied in the following ex-

Table VI

Influence of inhibitors of protein synthesis on the DNA synthesis in lymphocytes.
The effect of puromycin on protein synthesis was measured under conditions similar to those described in table I except that puromycin was added 39 h after concanavalin A. 1 h later the cells were isolated by centrifugation and resuspended in medium containing, in addition to mitogen, mercaptoethanol and puromycin, 5 μCi (^{3}H)leucine (specific radioactivity 50 Ci/mol). Incorporation was determined after 1 h. Control without puromycin: 4800 counts × min^{-1}. For measuring DNA synthesis the isolated cells were resuspended in medium as above except that (^{3}H)leucine was replaced by 1 μCi (^{3}H)thymidine. Incorporation was determined after 1 h. Control without puromycin: 6800 count × min^{-1}. Experiments with a 6 h incubation of drug: similar to those described in table III with betainyl-anguidine except that betainyl-anguidine or cycloheximide were added 38 h after concanavalin A. 4 h later 1 μCi (^{3}H)thymidine or 5 μCi (^{3}H)leucine were added and incorporation determined after 2 h. Controls without drug: incorporation of (^{3}H)leucine 15850 counts × min^{-1}; incorporation of (^{3}H)thymidine 20705 counts × min^{-1}. Experiments with cycloheximide or betainyl-anguidine (incubation with drug for 2 or 12 h resp.): similar to table III except that drug was added either 29 or 39 h after concanavalin A. 1 μCi(^{3}H)thymidine was added 40 h after mitogen and the incubation terminated after 1 h. Control without cycloheximide (2 h pulse): 4642 counts × min^{-1}; (12 h pulse): 11242 counts × min^{-1}; control without betainyl-anguidine (2 h pulse): 3972 counts × min^{-1}; (12 h pulse): 10886 counts × min^{-1}.

inhibitor	concentration (M)	period of incubation with drug (h)	percent inhibition of incorporation of	
			leucine	thymidine
puromycin	1,0 × 10^{-4}	2	93	87
cycloheximide	7,2 × 10^{-7}	6	86	70
betainyl-anguidine	0,2 × 10^{-7}	6	92	83
cycloheximide	1,8 × 10^{-7}	2	–	4
		12	–	67
betainyl-anguidine	0,2 × 10^{-7}	2	–	7
		12	–	85

periments: Cytosine arabinoside[2] inhibits DNA synthesis in stimulated lymphocytes. Simultaneously biosynthesis of histones but not of other proteins decreases strongly (Fig. 2). Similarly hydroxyurea[3] inhibits DNA synthesis in lymphocytes much more strongly than protein synthesis (table VII). Contrary to these inhibitors of DNA synthesis anguidine blocks protein synthesis more rapidly and more efficiently than DNA synthesis during a short incubation (table V). Therefore DNA synthesis is hardly the primary target of this drug.

If protein synthesis *in vivo* is the primary target of anguidine one would expect that this drug is also a very potent inhibitor of protein synthesis in a cell-free ribosomal system. Indeed, it has been demonstrated previously by many investigators that this classs of compounds inhibits initiation as well as elongation of protein synthesis on eukaryotic ribosomes in a cell-free

[2] an antimetabolite of biosynthesis of dCTP.

[3] a potent inhibitor of ribonucleoside diphosphate reductase, of an enzyme required for the synthesis of DNA precursors.

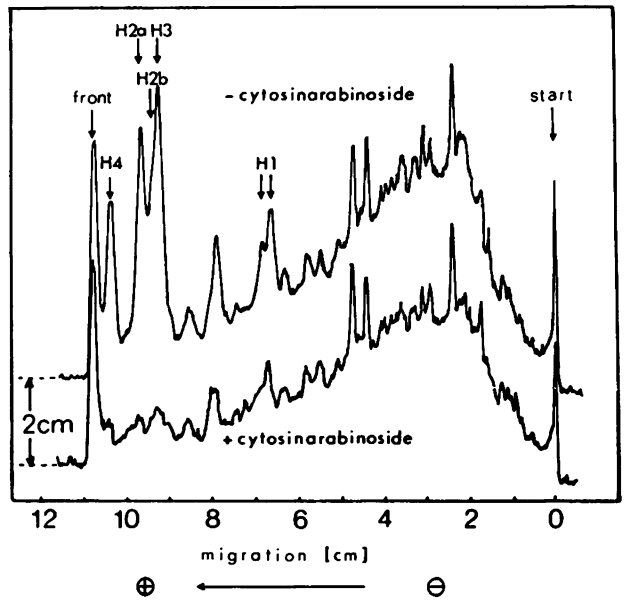


Fig. 2. Gel-electrophoretic analysis of radioactive labelled nuclear proteins synthesized during incubation of stimulated lymphocytes with (^{14}C)leucine in the presence or absence cytosine arabinoside.

Experiments were performed similar to Fig. 1 except that 20 μCi (^{14}C)leucine (specific radioactivity 309 Ci/mol) and 4.5×10^{-5} M cytosine arabinoside were added. After incubation nuclei were prepared as described [7] and dissolved in dodecylsulfate as in Fig. 1. To resolve the histones the gel system described [44] was used. Nuclear proteins [7] from 2×10^6 lymphocytes were applied to the gel.

Table VII

Influence of inhibitors of DNA synthesis on protein synthesis in lymphocytes.

Experiments were performed similarly to those described in table III except that the drug in 0.1 ml medium was added to 0.9 ml aliquots of stimulated lymphocytes 39 hours after mitogen. One hour later (^{3}H)thymidine or (^{3}H)leucine were added and incorporation into acid insoluble material measured after another 2 hours of incubation; controls without drug for DNA synthesis: 1) 14381 ± 1046 counts $\times$ min^{-1} (experiments with hydroxyurea); 2) 12240 ± 755 counts $\times$ min^{-1} (experiments with cytosine arabinoside). Controls without drug for protein synthesis: 1) 8917 ± 223 (experiments with hydroxyurea); 2) 6240 counts $\times$ min^{-1} (experiments with cytosine arabinoside).

inhibitor	concentration (M)	percent inhibition of incorporation of thymidine	leucine
hydroxyurea	$1{,}3 \times 10^{-4}$	77	10
	$13{,}0 \times 10^{-4}$	96	36
cytosine arabinoside	$4{,}1 \times 10^{-7}$	65	0
	$41{,}0 \times 10^{-7}$	91	10

Table VIII

Influence of inhibitors of protein synthesis in cell culture on the ribosomal synthesis of proteins in a cell-free system.

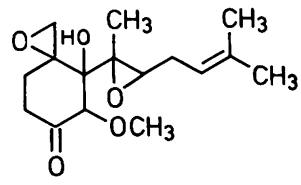
Experiments in cell culture: Essentially as described in table IV except that verrucarin A or betainyl-anguidine were added 39.5 h after concanavalin A and (^{3}H)leucine 30 min later. Incubation was terminated 1 h later. Experiments with cycloheximide were carried out similarly to the experiments in cell culture with betainyl-anguidine described in table III except that the drug was added 38 h after concanavalin A. 2 h later (^{3}H)leucine was added and the incubation continued for 2 more hours. *In vitro* experiments: essentially as described [40] with rat liver supernatant [41]. Ribosomes were prepared from lymphocytes stimulated for 42 h with concanavalin A (table III). Experiments with added mRNA: 50 μg poly rU and 1 μCi (^{3}H)phenylalanin (specific radioactivity 9 Ci/mmol); time of incubation 40 min except for cycloheximide (20 min); control without drug: about 4100 counts $\times$ min^{-1} in both experiments. In the experiments using endogenous mRNA 1 μCi (^{3}H)leucine was used as radioactive precursor; time of incubation: 20 min. Control without drug: 5195 counts $\times$ min^{-1}.

inhibitor	molar concentration required for 50 percent inhibition of protein synthesis		
	in cell culture	in a cell-free system	
		poly U	endogenous mRNA
verrucarin A	2×10^{-8}	2×10^{-5}	no effect at 2×10^{-5}
betainyl-anguidine	2×10^{-8}	2×10^{-5}	$> 2 \times 10^{-4}$
cycloheximide	4×10^{-7}	4×10^{-4}	1×10^{-3}

system [5, 6, 19–25]. It is rather striking, however, that usually more than 10^{-6} mol/l anguidine or verrucarin A are required for inhibition under these conditions. We have confirmed these observation for the inhibition of protein synthesis in a cell-free system with ribosomes from stimulated lymphocytes using either polyribouridylic acid or endogeneous RNA as messenger. To obtain 50% inhibition 2×10^{-5} mol/l of the toxin is required (table VIII). Obviously in this system protein synthesis is also much less sensitive to betainyl-anguidine than in cell culture. This unexpected discrepancy between the effect in cell culture and in a cell-free system has been observed with other inhibitors of protein synthesis such as cycloheximide [20, 26–28] and emetine [12, 28] in various eukaryotic cells and cellfree systems. We have confirmed the observation with cycloheximide in stimulated lymphocytes (table VIII). It may be explained by assuming the presence or absence of a soluble protein factor which determines the sensitivity to the inhibitors. On the basis of this assumption the concentration of such a soluble factor would control the inhibitory activity of the drugs. Indeed, such a soluble protein has been isolated from the supernatant of a yeast lysate which renders sensitivity towards cycloheximide to the cell-free system from yeast [29].

II. Ovalicin

Ovalicin has been isolated from the culture medium of the ascomycete *Pseud-*



Ovalicin

eurotium ovalis STOLK by SIGG and WEBER. The same authors have determined its chemical structure [30]. Its immunosuppressive activity *in vivo* deserves particular attention: the number of antibody producing cells in the spleen of mice immunized with sheep red blood cells decreases to less than one per cent if 600 mg ovalicin per kg mouse is injected one day after immunisation. With later injection the inhibitory effect declines rapidly. Similarly the graft versus host reaction is distinctly delayed by ovalicin. The drug reduces the number of mitoses in spleen cells of immunised mice whereas the mitotic index in the jejunum is unchanged [31, 32]. These observations suggested a study of the action of ovalicin on cells of the lymphatic system. Following our proposal SCHIMPL and WECKER have investigated the effect of the drug on the induction of antibody production in cultures of mouse spleen cells [33]. Surprisingly the activity of ovalicin in this system turned out to be much higher than in the animal. Even at a concentration of 4×10^{-9} mol/l a distinct inhibition was observed [34]. One reason for the apparently lower activity in the animal may be the much higher metabolic turnover rate of the drug.

We decided to analyse the mode of action of ovalicin by measuring a more convenient reaction in a lympocyte cell culture system. For this purpose we have chosen thymidine incorporation into DNA as an indicator of cell proliferation in lymphocytes stimulated by mitogen. If ovalicin is added together with concanavalin A to murine splenic lymphocytes incorporation of radioactively labelled thymidine into DNA during the S-phase is strongly reduced. Even at a concentration of 2×10^{-10} mol/l ovalicin a 50% decrease of thymidine incorporation is observed (table III). However, at much higher concentrations of the drug inhibition of DNA synthesis is by no means complete. The extent of this ovalicin resistant thymidine incorporation depends on many factors such as the concentration of the mitogen, cell density, presence of mercaptoethanol or time of measurement of DNA synthesis.

Ovalicin is not only active on DNA synthesis induced artificially by plant lectins such as concanavalin A, purified phytohaemagglutinin or poke weed mitogen (data not shown). In a mixed lymphocyte culture proliferation is induced by the presence of several different antigenic determinants on the cell surface of the allogeneic lymphocytes. In this system ovalicin also is strongly inhibitory to DNA synthesis (table IX).

All these observations demonstrate that ovalicin is one of the most potent low-molecular inhibitors of proliferation of lymphocytes.

A suspension of spleen cells contains populations of many different cells, among them lymphocytes of B and T type. Therefore the question arises if ovalicin inhibits proliferation only in certain types of cells. B and T cells may

Table IX

Influence of ovalicin on the thymidine incorporation into proliferating lymphocytes.

Mixed lymphocyte culture: A mixture of 5×10^5 spleen lymphocytes each from C 57 Bl/6 and DBA/2 mice were incubated at 37° C with or without drug in Eagle's MEM containing 5 % fetal calf serum [38] (total volume 1 ml). After 48 h μCi (^{3}H)thymidine (specific radioactivity 10 Ci/mmol) were added and the incubation continued for 6 h.

Lymphoblastoma cells: Exponentially growing cells were cultured at 37° C in Dulbecco's modified Eagle's medium (without nonessential amino acids) containing 10 % fetal calf serum (total volume 15 ml) with or without drug. After 48 h 0.9 ml aliquots were transferred to test tubes, 1 μCi (^{3}H)thymidine (specific radioactivity 0.5 Ci/mmol) in 0.1 ml was added and the incubation continued for 1 h.

Spleen cells (athymic nu/nu mouse): 10^6 cells were incubated in the presence of 2 μg lipopolysaccharide and ovalicin for 36 h (total volume 1 ml). Subsequently 1 μCi (^{3}H)thymidine was added and the incubation continued for 24 h.

Human peripheral blood lymphocytes: lymphocytes from fresh human blood (42) were treated as described under spleen cells except that 5 μg/ml Concanavalin A was used as mitogen.

type of cell	ovalicin (mol/l)	incorporation of [^{3}H] thymidine (counts $\times$ min^{-1})		inhibition (%)
		+ ovalicin	— ovalicin	
mixed mouse lymphocytes (C 57 Bl/6 + DBA 2)	$0{,}3 \times 10^{-11}$	2538 ± 917	6471 ± 1305	61
S 49.1 lymphoblastoma	$1{,}0 \times 10^{-9}$	35410 ± 760	70820 ± 340	50
spleen cells (athymic nu/nu mouse)	$3{,}0 \times 10^{-7}$	4609 ± 343	13557 ± 1213	65
peripheral human lymphocytes	$2{,}0 \times 10^{-7}$	1971 ± 260	3131 ± 226	37

be stimulated separately by various mitogens such as concanavalin A which induces only T cells whereas lipopolysaccharide from the outer membrane of gram-negative bacteria induces only B cells [35]. In each case the proliferation induced by these type-specific mitogens is inhibited by very small concentrations of ovalicin (table IX). This is also true for the mitogen induced proliferation of human peripheral lymphocytes (table IX). Similarly, thymidine incorporation in transformed lymphocytes such as monoclonal S 49.1 mouse lymphoma cells [36] is strongly reduced after 48 h incubation with the toxin (table IX). This observation demonstrates the direct action of ovalicin on lymphocytes. Its action is not mediated by cell-cell interaction, for example via macrophages.

The inhibitory action of ovalicin on cells different from those of the lymphatic system has also been studied. Thymidine incorporation in 3T6 mouse fibroblasts or HeLa cells is distinctly less inhibited by ovalicin. Incubation with 2×10^{-7} mol/l drug for two days results in only 30–40 % inhibition whereas at this concentration inhibition in S 49.1 lymphoma cells is 80 % at this concentration. However, the smaller inhibitory effect on 3T6 or HeLa cells is already detectable at the low drug concentration of 2×10^{-9} mol/l (data not shown). These observations suggest

that lymphocytes are more susceptible to the action of ovalicin than other cells, in agreement with observations on the effects of the drug on the various animal tissues [31].

In the experiments reported so far incorporation of the nucleoside thymidine into DNA was used as analytical tool to demonstrate the activity of ovalicin. If DNA synthesis is directly affected the drug should be able to block the incorporation of deoxyribonucleoside triphosphates into DNA in a cell free system from lymphocytes [7]. However, even at concentration of 3×10^{-6} mol/l ovalicin does not act inhibitory in this system (table III). Obviously the polycondensation of precursors to DNA is not the target of the drug. On the other hand DNA synthesis is clearly reduced in a cell-free system prepared from lymphocytes which had been preincubated with ovalicin compared with the activity of a system from untreated cells (table X). This observation supports the notion that ovalicin inhibits the formation of an essential element of the DNA synthesizing machinery.

If this notion is correct it is be expected that the time of addition of the inhibitor to the cell culture should clearly influence the extent of inhibition. Indeed, this notion has been confirmed. If ovalicin is added to a spleen cell culture at various times after mitogenic stimulation maximal inhibition is observed only when the drug is added until the 6th to the 8th hour after mitogen. If added 16–20 h after mitogen no effect is measured on the DNA synthesis measured 14 h later (Fig. 3). Obviously the early phase of lymphocyte stimulation is particularly sensitive to the inhibitor.

On the other hand, characteristic reactions occurring immediately after addition of the mitogen are not at all reduced by the drug. This conclusion may be derived from the observation that ovalicin added 6 h after the mitogen shows its full inhibitory activity (Fig. 3). The same conclusion is derived from the investigation of the lipid

Table X

Influence of ovalicin on the incorporation of (^3H)dTTP into DNA of nuclei of lymphocytes. The experiment was performed as described in table III except that nuclei were prepared from lymphocytes (2×10^6 cells/ml) stimulated for 46 h with 1 μg/ml concanavalin A in the absence of mercaptoethanol; concentration of ovalicin: 7×10^{-7} mol/l.

pretreatment of lymphocytes	[^{3}H] dTTP incorporation into nuclei (counts $\times$ min^{-1} $\times$ 10^{-6} nuclei)
+ Con A + ovalicin	115
+ Con A — ovalicin	231
— Con A — ovalicin	31

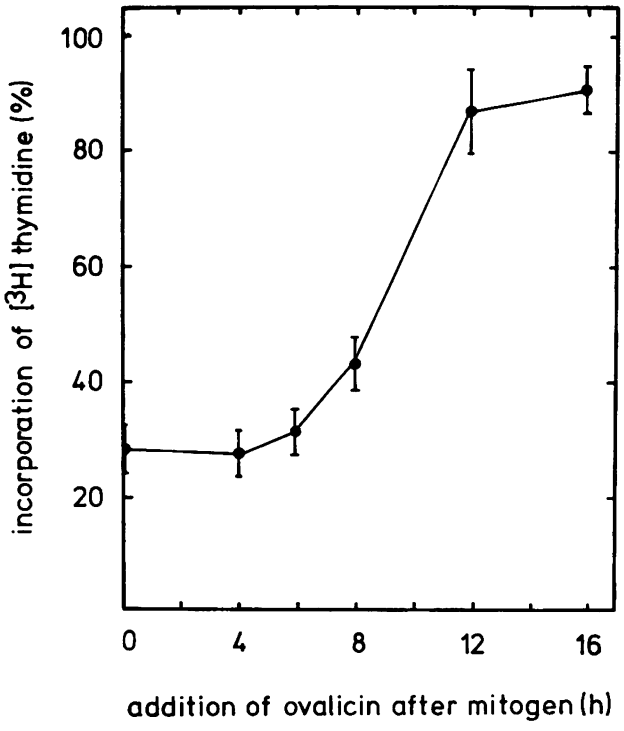


Fig. 3. Influenceof the time of addition of ovalicin on (^3H)thymidine incorporation into DNA of stimulated lymphocytes.

1 μg/ml concanavalin A was added to 10^6 lymphocytes (preincubated for 12 h at 37° C). 2×10^{-7} M ovalicin were added after the time indicated on the abscissa. 30 h after mitogen (^3H)thymidine was added and the incubation continued for 6 h. Controls without ovalicin: 21835 ± 1094 counts × min^{-1} = 100%.

turnover which, in lymphocyte membranes, is significantly increased very soon after addition of mitogen [37]. The lipid turnover is increased also in presence of ovalicin (table XI).

These observations may lead to the conclusion that ovalicin acts on stimulated lymphocytes only during a very short phase of the cell cycle (6–12 h after addition of mitogen). However, this conclusion could not be confirmed. Rather a 14–16 h incubation period of the drug is required for maximum inhibition of stimulated spleen lymphocytes from mouse as is shown by the following experiments: in the first experiment the drug was added to the cell culture various times after the mitogen. 20 h after addition of the mitogen the inhibitor was removed from all samples by repeated washing (Fig. 4 (●)). The strongest inhibition of DNA synthesis was observed when ovalicin had acted on the cells for at least 16 h. In the second experiment the drug was added uniformly to all samples 6 h after mitogen. It was then removed from the samples after 2–14 h incubation (Fig. 4 (▲)). As in the previous experiment an incubation period of at

Table XI
Influence of ovalicin on the incorporation of (^{14}C)acetate into lecithin from lymphocytes.
Lymphocytes from spleen (2×10^6 cells/ml, preincubated for 15 h) were incubated in Eagle's MEM containing 2.5 % calf serum, 1 μg/ml concanavalin A and 10 μmol/l mercaptoethanol. Incorporation of (^{14}C) acetate (56 Ci/mol) into lecithin was measured essentially as described [37]. Concentration of ovalicin: 2×10^{-7} mol/l (added with Con A).

additions	incorporation of [^{14}C]acetate into lecithin from lymphocytes between 0–4 h after addition of Con A (counts × min^{-1})	lecithin content (in % of total lipids)
+ Con A + ovalicin	904	15.6
+ Con A — ovalicin	803	14.3
— Con A — ovalicin	567	13.5

least 14 h is required to obtain the strongest inhibitory effect. This long incubation period may be required for several reasons: existence of various populations of cells in the culture, a very slow permeation of the drug into the cells or a metabolic activation of ovalicin.

However we were unable to detect any metabolic products in the medium of the cell culture after a long incubation with the drug which would inhibit stimulated lymphocytes with higher activity (data not shown).

The preceding experiments show that the cell cultures have to be incubated with ovalicin for a rather long period of time to obtain a maximal inhibitory effect. But at which time can the first effect of the drug be observed? If the rate of DNA synthesis is measured using short pulses of radioactively labelled thymidine and starting immediately after addition of mitogen, an incorporation of thymidine into DNA is observed as soon as 7–8 h after mitogen (Fig. 5). Although exceedingly small, it is clearly higher than that observed in a cell culture not having received mitogen. If 2×10^{-7} mol/l ovalicin are added together with the mitogen even this very early incorporation is distinctly inhibited (Fig. 5). Again the effect of ovalicin is not observed immediately after addition but requires an incubation period of several hours (data not shown).

All these observations are at variance with DNA synthesis as a direct target of the drug. Therefore the action of the toxin on the biosynthesis of other macromolecules has also been investigated.

Incorporation of radioactively labelled uridine into the acid insoluble material of cell lysate was used to assay for the biosynthesis of RNA. Incorporation of uridine into RNA is significant even in resting lymphocytes. It is

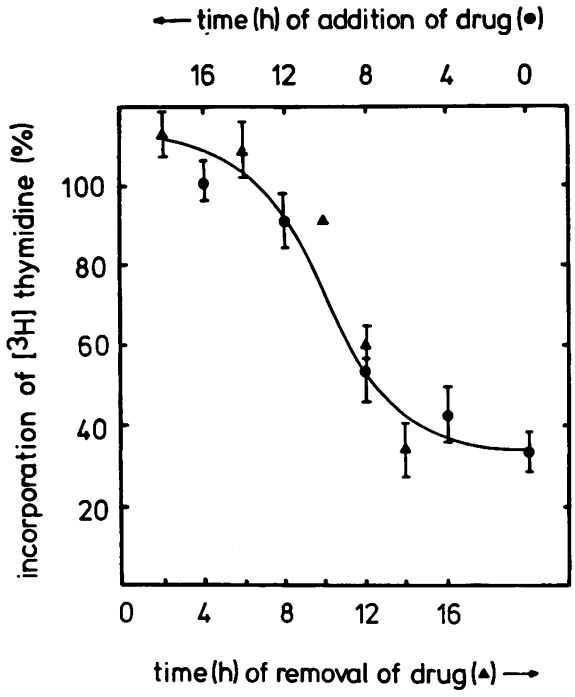


Fig. 4. Influence of the length of incubation period of lymphocytes with ovalicin on the inhibition of DNA synthesis.
1 μg ConA was added to 10^6 lymphocytes (preincubated for 12 h at 37° C) in 1 ml medium. In one series of experiments (●) 3×10^{-9} M ovalicin were added after addition of ConA at the time indicated on the upper abscissa and the drug removed 20 h after addition of mitogen by sedimenting of the cells and resuspending in fresh medium. This washing procedure was repeated twice. Controls were treated similarly. 25 h after addition of mitogen 1 μCi (^{3}H)thymidine was added and the incubation continued for 11 h. In a second series of experiments (▲) 3×10^{-9} M ovalicin were added 6 h after mitogen and removed by repeated washings after the times indicated on the lower abscissa. Controls without drug: 29198 ± 2689 counts × min^{-1} = 100%.

not reduced even by 3×10^{-7} mol/l ovalicin. In lymphocytes stimulated with concanavalin A RNA synthesis is only weakly inhibited after 20 h incubation with the drug (Table XII). Obviously, RNA synthesis as followed by total incorporation of a precursor, is not the target of the drug.

Furthermore, this observation also indicates that the production of energy in lymphocytes is not the target of ovalicin because RNA synthesis is strongly dependent on this process.

In eukaryotic cells, DNA synthesis is tightly coupled to protein synthesis as has been discussed in the section on anguidine. Therefore the influence of ovalicin on protein synthesis has been investigated. The incorporation of radioactively labelled leucine into the acid insoluble material of cell lysates was used to assay for protein biosynthesis. 14 h incubation with 2×10^{-7} mol/l ovalicin leads to a 20% reduction of protein synthesis whereas the small extent of DNA synthesis is already redu-

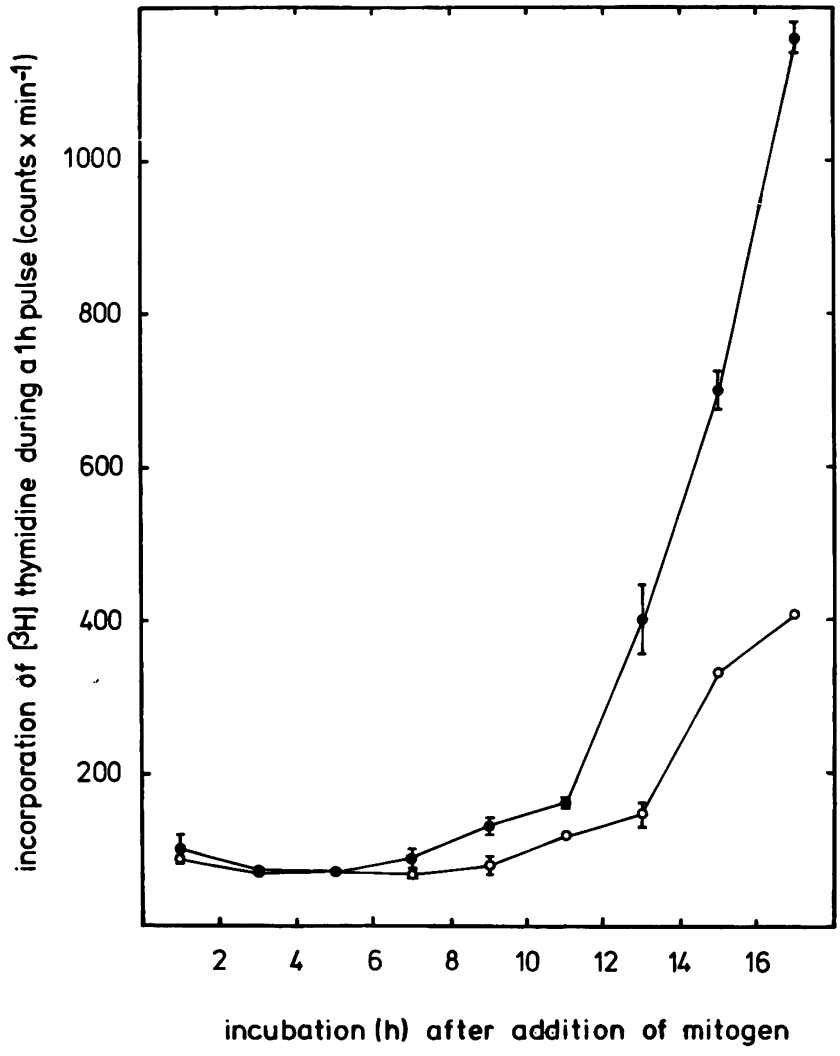


Fig. 5. Influence of ovalicin on DNA synthesis in lymphocytes observed shortly after addition of mitogen.
Lymphocytes were stimulated as described in table XI. Incorporation of (^{3}H)thymidine (0.5 Ci/mmol, 1 μCi/ml) during a one hour pulse was measured; concentration of ovalicin used: 2×10^{-7} mol/l.

ced by 60–70% at this early phase of the cell cycle (table XIII). The inhibition of protein synthesis is increased to 40% when the incubation period with the drug is extended to 40 h (table XIII). This increased extent of inhibition may be the consequence of the blocked biosynthesis of DNA as has been discussed above. In this context we wish to mention the observation that the elongation process of protein biosynthesis as measured with ribosomes from stimulated

Table XII

Influence of ovalicin on the incorporation of (^{14}C)uridine into RNA of stimulated lymphocytes. 1 μg concanavalin A/ml and 3×10^{-7} mol/l ovalicin were added to 10^6 lymphocytes/ml (preincubated for 12 h at 37° C). After 20 h 80 nCi (^{14}C)uridine (specific radioactivity 414 Ci/mol) were added and the incubation continued for 1 h.

additions	incorporation of [^{14}C] uridine during a one hour pulse (counts × min^{-1})
+ Con A + ovalicin	2651
+ Con A — ovalicin	3419
— Con A + ovalicin	1170
— Con A — ovalicin	1186

Table XIII

Inhibition by ovalicin of the incorporation of (^{3}H)leucine or (^{3}H)thymidine into acid insoluble material of stimulated lymphocytes.

Preincubated lymphocytes were stimulated essentially as described in table XI. Protein synthesis was followed by incorporation of (^{3}H)leucine (specific radioactivity 57 Ci/mol when added 12 h after mitogen, 13 Ci/mol when added 36 h after mitogen) during a 2 h pulse essentially as described in table I. Incorporation of (^{3}H)thymidine (specific radioactivity 0.5 Ci/mol) during a 2 h pulse was determined essentially as described in table III. Concentration of ovalicin: 2×10^{-7} mol/l (added with Con A).

additions	time of addition of radioactive precursor	incorporation of [^{3}H] leucine (counts × min^{-1})	incorporation of [^{3}H] thymidine (counts × min^{-1})
+ ovalicin	12	5700 ± 139	443 ± 30
— ovalicin	12	7741 ± 27	1094 ± 28
+ ovalicin	36	4391 ± 62	13250 ± 934
— ovalicin	36	8565 ± 308	40594 ± 1664

lymphocytes in a cell-free system is not inhibited by 3×10^{-7} mol/l ovalicin (table XIV).

If the inhibition of protein biosynthesis by the toxin is the direct consequence of the inhibition of DNA synthesis the formation of the histones should be affected particularly as has been shown above for cytosine arabinoside by electrophoretic analysis (Fig. 2). However, this is not found. In the presence of ovalicin the formation of all proteins is uniformly reduced (data not shown).

Summarizing the numerous observations reported we have to conclude that neither DNA nor protein synthesis is a direct target of ovalicin. This notion is

Table XIV
Effect of ovalicin on the poly rU directed synthesis of polyphenylalanine in a cell-free system with ribosomes from stimulated lymphocytes.
Ribosomes were prepared from lymphocytes stimulated as described in table XI except that mercaptoethanol was added 23 h after addition of mitogen. The experiments with the cell-free system were performed as in table VIII except that the time of incubation was 15 min.

additions	incorporation of [^{3}H] phenylalanine (counts × min^{-1})
no	2630 ± 494
+ 0.7×10^{-6} mol/l ovalicin	2707 ± 310
+ 1×10^{-5} mol/l ovalicin	2545 ± 293
without ribosomes	1040 ± 271

supported by a comparison of the action of ovalicin with that of cycloheximide or cytosine arabinoside on the transformation of small resting lymphocytes into large blast cells. After addition of the mitogen to a cell culture the average diameter of the cells increases up to the 48th hour of incubation as measured in the microscope. If cycloheximide is added together with the mitogen the average diameter of the lymphocytes remains unchanged as in the absence of mitogen. Obviously continuous protein synthesis is required for blast cell formation. In the presence of cytosine arabinoside the average cell diameter increases up to the 24th hour almost as much as in the absence of this specific inhibitor of DNA synthesis. During the following day no further increase is observed. The effect of ovalicin is very different from that of the drugs mentioned above. Here the formation of blast cells continues up to two days of incubation although it is distinctly slower than in the absence of ovalicin (Fig. 6). In contrast DNA synthesis, as measured simultaneously by incorporation of thymidine into the acid insoluble material of the cells, is inhibited very extensively (Fig. 6). These oberservations indicate that the mechanism of action of ovalicin is very different from that of specific inhibitors of protein and DNA synthesis. Further investigations to pinpoint the target will be required. They promise to be rewarding since they will reveal the reason for the exceptionally high sensitivity of lymphocytes to the toxin as well as a key reaction crucial for lymphocyte proliferation, since otherwise 10^{-10} mol/l ovalicin would not be expected to act inhibitory.

Acknowledgements

These investigations have been supported in part by the Sonderforschungsbereich 105 (Cytologische Grundlagen der experimentellen Biologie) and by the Fonds der Chemischen Industrie. The toxins used have been kindly provided by Drs. H.-P. Sigg and H. Staehelin, Sandoz AG,

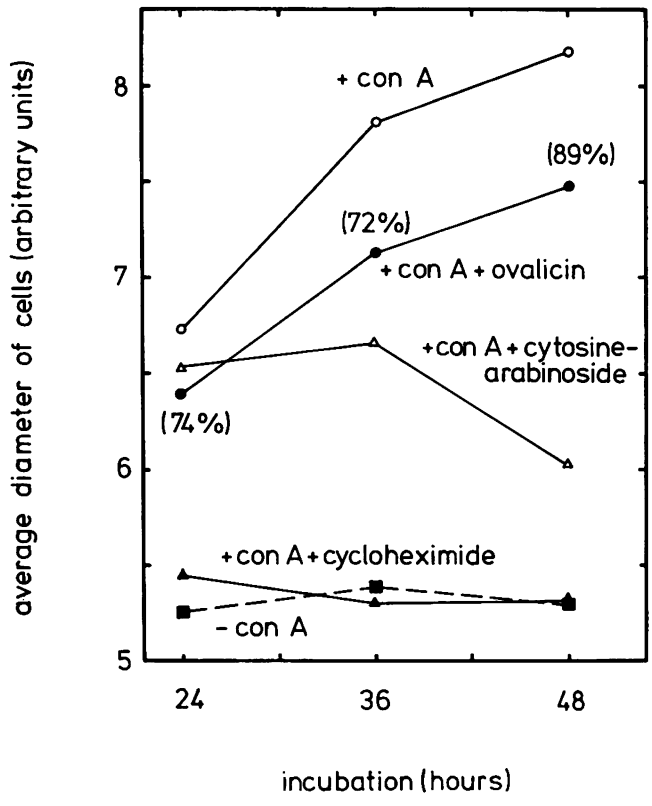


Fig. 6. Inhibition of the transformation of small lymphocytes into blast cells by ovalicin, cytosine arabinoside or cycloheximide.

Lymphocytes were stimulated as described in table XI. Drugs were added togehter with the mitogen: 2×10^{-7} M ovalicin, 1×10^{-6} M cycloheximide or 1×10^{-5} M cytosine arabinoside, respectively. About 5×10^{5} washed cells (in 0.25 ml) were mixed with 0.5 ml warm 0.5% agarose in phosphate-buffered saline, and applied to slides. The coated slides were treated with 0.5% glutaraldehyde in phosphate-buffered saline, washed three times for 5 min in destilled water, air-dried and Giemsastained [45]. The diameter of the stained cells was estimated at a magnification of 1:1250 in arbitrary units and the average diameter of 250 cells calculated. DNA synthesis (values in per cent are given in brackets) at 24, 36 and 48 h of incubation after addition of concanavalin A was determined by an one hour (^{3}H)thymidine pulse (1 μCi/ml; 0.5 Ci/mmol) in 0.9 ml aliquots of the cell culture. 100% of DNA synthesis at 24, 36 and 48 h respectively: 19957 ± 84, 50285 ± 3286, 116805 ± 222 counts $\times$ min^{-1}, respectively. In the presence of cycloheximide or cytosine arabinoside the incorporation of thymidine into the acid-insoluble material of the cells is neglegible.

and by Dr. C. Tamm, Institut für Organische Chemie, University of Basel. The first investigations on the mode of action of betainyl-anguidine were performed by Dr. T. Neudecker in our laboratory. We wish to express our deep gratitude to Drs. A. Schimpl, E. Wecker and C. Jungwirth, Institut für Virologie und Immunbiologie, University of Würzburg, for advice, suggestions and many helpful discussions and for providing us with informations about unpublished experiments. We are obliged to Dr. U. Gehring, Institut für Biologische Chemie, University of Heidelberg, for providing us with lymphoblastoma cells and for advice to grow them in cell culture and to Dr. E.-L. Winnakker and his group, Institut für Biochemie, University of München, for help in the experiments with HeLa and 3T6 cells.

References

1. Tamm, Ch.: Fortschr. Chem. org. Naturst. *31*, 63 (1974).
2. Staehelin, H., M. E. Kalberer-Rüsch, E. Signer and S. Lazáry: Arzneim. Forsch. *18*, 989 (1968).
3. Hartmann, G., K. O. Honikel, F. Knüsel and J. Nüesch: Biochim. Biophys. Acta *145*, 843 (1967).
4. Moss, B. and E. N. Rosenblum: J. Mol. Biol. *81*, 267 (1973).
5. Ueno, Y. and N. Shimada: Chem. Pharm. Bull. *22*, 2744 (1974).
6. Liao, L.-L., A. P. Grollman and B. B. Horwitz: Biochem. Biophys. Acta *454*, 273 (1976).
7. Benz, W. C. and J. L. Strominger: Proc. Natl. Acad. Sci. USA *72*, 2413 (1975).
8. Powell, W. R.: Biochim. Biophys. Acta *55*, 979 (1962).
9. Mueller, G. C., K. Kajiwara, E. Stubblefield and R. R. Rueckert: Cancer Res. *22*, 1084 (1962).
10. Kerridge, D.: J. Gen. Microbiol. *19*, 497 (1958).
11. Bennett, L. L. jr., D. Smithers and C. T. Ward: Biochim. Biophys. Acta *87*, 60 (1964).
12. Grollman, A. P.: J. Biol. Chem. *243*, 4089 (1968).
13. Tyrsted, G., B. Munch-Petersen and L. Cloos: Exptl. Cell Res. *77*, 415 (1973).
14. Stimac, E., D. Housman and J. A. Huberman: J. Mol. Biol. *115*, 485 (1977).
15. Venkatesan, N.: Biochim. Biophys. Acta *478*, 437 (1977).
16. Weintraub, H.: Nature *240*, 449 (1972).
17. Elgin, S. C. R. and Weintraub, H.: Ann. Rev. Biochem. *44*, 725 (1975).
18. Weintraub, H.: Cell *9*, 419 (1976).
19. Ohtsubo, K. and M. Saito: Japan. J. Med. Sci. Biol. *23*, 217 (1970).
20. Ueno, Y., M. Nakajima, K. Sakai, K. Ishii, N. Sato and N. Shimada: J. Biochem. (Tokyo) *74*, 285 (1973).
21. Schindler, D.: Nature *249*, 38 (1974).
22. Schindler, D., P. Grant and J. Davies: Nature *248*, 535 (1974).
23. Wei, C. M. and C. S. McLaughlin: Biochem. Biophys. Res. Commun. *57*, 838 (1974).
24. *Mizuno, S.:* Biochim. Biophys. Acta 383, 207 (1975).
25. Jimenez, A., L. Sanchez and D. Vazquez: Biochim. Biophys. Acta *383*, 427 (1975).
26. Wescott, E. W. an H. D. Sisler: Phytopathology *54*, 1261 (1964).
27. Colombo, B., L. Felicetti and C. Baglioni: Biochem. Biophys. Res. Commun. *18*, 389 (1965).
28. Entner, N. and A. P. Grollman: J. Protozool. *20*, 160 (1973).
29. Somasundaran, U. and L. Skogerson: Biochemistry *15*, 4760 (1976).
30. Sigg, H. P. and H. P. Weber: Helv. chim. Acta *51*, 1395 (1968).
31. Lazáry, S. and H. Staehelin: Experientia *24*, 1171 (1968).
32. Lazáry, S. and H. Staehelin: Antibiotica Chemother. *15*, 177 (1969).
33. Mishell, R. I. and R. W. Dutton: J. Exptl. Med. *126*, 423 (1967).
34. Schimpl, A. and E. Wecker: University of Würzburg, unpublished experiments (1972).
35. Andersson, J., G. Möller and O. Sjöberg: Cell. Immunol. *4*, 381 (1972).
36. Harries, A. W.: Exptl. Cell Res. *60*, 341 (1970).
37. Resch, K. and E. Ferber: Eur. J. Biochem. *27*, 153 (1972).
38. Dörries, R., A. Schimpl and E. Wecker: Eur. J. Immunol. *4*, 230 (1974).
39. Waithe, W. J., P. Hathaway and K. Hirschhorn: Clin. exp. Immunol. *9*, 903 (1971).
40. Ahern, T. and J. E. Kay: Biochim. Biophys. Acta *331*, 91 (1973).
41. Sampson, J. and A. F. Borghetti: Nature New Biol. *238*, 200 (1972).
42. Valentine, F. in Bloom, B. R. and P. R. Glade (eds.): In vitro Methods in Cell Mediated Immunity, p. 443, New York-London, 1971, Academic Press.
43. Laemmli, U. K.: Nature *227*, 680 (1970).
44. Thomas, J. O. and R. D. Kornberg: Proc. Natl. Acad. Sci. USA *72*, 2626 (1975).
45. Hünig, T., A. Schimpl and E. Wecker: J. Exp. Med. *139*, 754 (1974).

Address: Prof. Dr. G. R. Hartmann
Institut für Biochemie
Karlstr. 23
D-8000 München 2