Eudistomins A–Q, β-Carbolines from the Antiviral Caribbean Tunicate *Eudistoma olivaceum*[1]

Kenneth L. Rinehart, Jr.,*[2a] Jun'ichi Kobayashi,[2a] Gary C. Harbour,[2a] Jeremy Gilmore,[2a] Mark Mascal,[2a] Tom G. Holt,[2a] Lois S. Shield,[2a] and Françoise Lafargue[2b]

Contribution from the Roger Adams Laboratory, University of Illinois at Urbana—Champaign, Urbana, Illinois 61801, and the Laboratoire Arago, Université de Paris VI, Banyuls-sur-mer, France. *Received January 10, 1986. Revised Manuscript Received January 26, 1987*

Abstract: Seventeen eudistomins have been isolated from the Caribbean colonial tunicate *Eudistoma olivaceum*. Twelve are β-carbolines—four of them (eudistomins D, J, N, and O) unsubstituted at C-1, three (A, B, and M) with pyrrol-2-yl substituents at C-1, and five (G, H, I, P, and Q) with 1-pyrrolin-2-yl substituents at C-1; five (eudistomins C, E, F, K, and L) are 1,2,3,4-tetrahydro-β-carbolines with an oxathiazepine ring fused at C-1 and N-2. Syntheses are described of eudistomins D, H, I, M, N, O, and Q and of two related compounds. The major route to both 1-(pyrrol-2-yl)- and 1-(1-pyrrolin-2-yl)-substituted eudistomins proceeded through Grignard addition of 2-(1,3-dioxa-2-cyclohexyl)ethyl bromide to 1-cyano-substituted β-carbolines, followed by appropriate cyclization, reduction, and dehydrogenation.

Eudistomins A–Q[1] (**1–17**, respectively; Chart I) were extracted from the colonial tunicate *Eudistoma olivaceum*, collected in shallow water in Mexico,[3] Belize, and Florida. Crude extracts of all the *E. olivaceum* samples collected inhibited plaque formation by *Herpes simplex* virus, type 1 (HSV-1), in CV-1 cells (monkey kidney tissue) with little cytotoxicity at the level tested. During the isolation sequence (see Experimental Section and scheme in supplementary material),[1a,b] *E. olivaceum* fractions were assayed continuously for antiviral activity, which was found mainly in the chloroform layer, with second and third loci of activity in the 1-butanol and toluene layers.[1c] Work thus far has centered on the toluene and chloroform layers, from which 17 bioactive β-carbolines have been isolated, some of them with promising antiviral activity; the pyrrolinyl- and pyrrolyl-substituted β-carbolines are least polar, the oxathiazepines next, and the unsubstituted β-carbolines most polar. Structures **1–17** were assigned by spectroscopic techniques (see Tables I–III and Figures 1 and 2 in supplementary material),[1a,b] including high-resolution fast atom bombardment (HRFAB) and HR electron ionization mass spectrometry (EIMS) and 500- and 360-MHz ^1H and ^{13}C NMR spectroscopy, and by the syntheses of eudistomins D, H, I, M, N, O, and Q and of two related neoeudistomins, **18** and **19** (Chart I), as model compounds.

Eudistomins D, J, N, and O. Structures **14** and **15**, assigned to eudistomins N and O, respectively, on the basis of UV and NMR spectroscopy[1a,b,4] and FABMS,[1a,b] were confirmed by synthesis, as was structure **4**, assigned to eudistomin D from spectral data.[1a,b,4] Eudistomin J was isolated as a 1:1 mixture with the isomeric eudistomin D, but the ^1H NMR spectrum[4] was well resolved. Diacetyleudistomin J (**20**, Chart II) was separated from the isomeric diacetyleudistomin D (**22**, Chart II) and assigned structure **20** from its ^1H NMR spectrum (with two benzenoid singlets) relative to that of **22**.

The preparation of eudistomins N and D was relatively facile (Scheme I). Bromination of β-carboline gave eudistomin N (**14**). Synthesis of eudistomin D (**4**) involved bromination of 6-methoxy-β-carboline[5,6] [prepared from commercially available 5-methoxytryptamine by the usual tryptamine → β-carboline route (glyoxylic acid condensation, decarboxylation, dehydrogenation)] and demethylation of the resulting 5-bromo-6-methoxy-β-carboline (**29**).

A considerably more tortuous route was required for eudistomin O, starting from 4-amino-2-nitrotoluene (Scheme II). An alternative route to **31** employed the Fischer indole synthesis involving 3-bromophenylhydrazine and 4-aminobutanal diethyl acetal (not shown). The latter, Fischer route provided a direct, one-step synthesis of **31**, proceeding in 60% yield to give a mixture of the desired 6-bromo (**31**, 36%) and 4-bromo (24%) isomers, which could be separated efficiently on a preparative scale with

Chart I

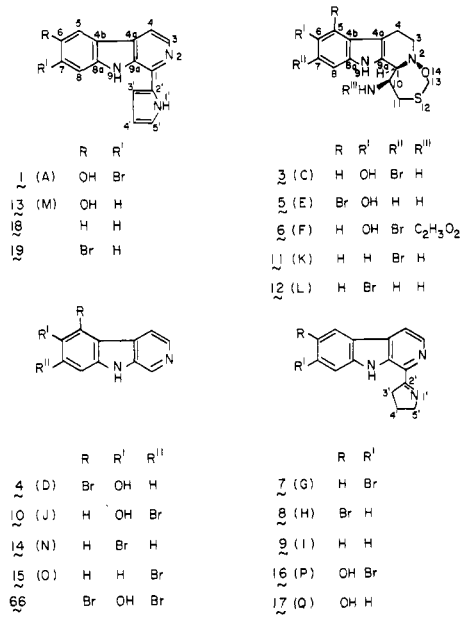
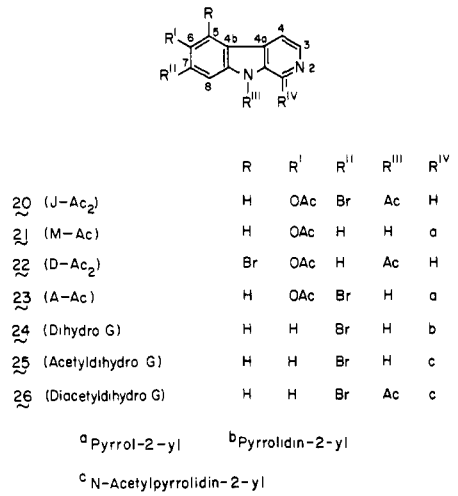


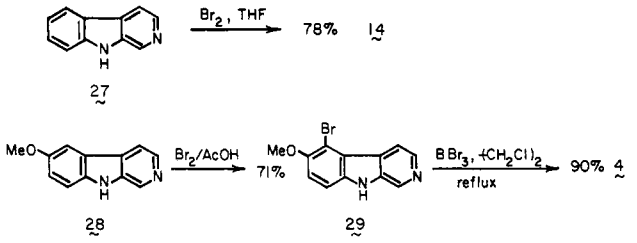
Chart II



* Address correspondence to this author.

0002-7863/87/1509-3378$01.50/0

Scheme I



β-cyclodextrin reversed-phase HPLC. Conversion of the tetrahydro-β-carboline **32** to eudistomin O (**15**) by an alternative oxidation, employing triphenylmethyl trifluoroacetate[7] instead of diphenylselenium bis(trifluoroacetate), gave a far lower yield (16%) of **15**, and glyoxylation of **30** followed by reduction yielded debrominated products. The UV spectra and ^{1}H NMR peak patterns of synthetic eudistomins D, N, and O agreed with those of the natural products.[4]

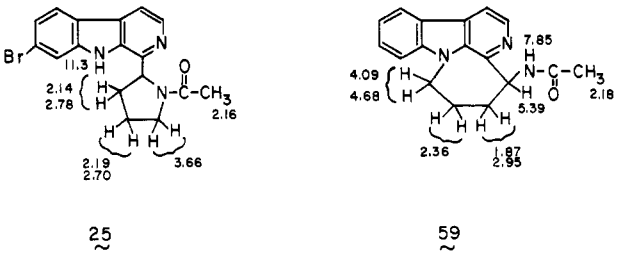
Eudistomins A, B, and M. Eudistomins A and M (**1** and **13**) contain the β-carboline ring system substituted by a pyrrol-2-yl group at C-1. Eudistomin M was isolated as its acetate, whose structure was assigned as **21** (Chart II) from UV and NMR data and FABMS.[1a,b,4] The acetoxyl group was located at C-6 (or C-7) by the ^{1}H NMR coupling pattern (ortho, meta) of the benzenoid protons and at C-6 by the coupling constant. The decision in favor of C-6 was supported by analogy to eudistomins D and J, and structure **13** was confirmed by synthesis.

Acetyleudistomin A (**23**, Chart II), prepared from eudistomin A, gave UV and NMR data[4] consistent with the assignment of a pyrrolyl-substituted β-carboline ring system and with data for acetyleudistomin M. Additional support for structure **1** derives from ^{13}C NMR spectroscopy.[1a,b,4]

Syntheses of 1-(pyrrol-2-yl) eudistomin M and of neoeudistomins 1 and 2 were accomplished by using 1-cyano-β-carboline (**41**, Scheme III) as a common intermediate, with subsequent addition of a difunctional three-carbon unit and cyclization to the appropriate five-membered heterocyclic rings. The preparation of **41** from 1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid[5] involved esterification, conversion to the carboxamide **37**, catalytic dehydrogenation, and dehydration.

The required three-carbon unit, with functionalities at both the 1- and 3-positions, was provided by the Grignard reagent of 2-(1,3-dioxa-2-cyclohexyl)ethyl bromide,[8] which reacted with **41** in tetrahydrofuran (THF); mild acid hydrolysis of the intermediate gave 1-[3-(1,3-dioxa-2-cyclohexyl)propanoyl]-β-carboline (**44**, Scheme IV). Cyclization of **44** by heating with ammonium acetate in acetic acid[9] afforded the pyrrole **18**, a synthetic member of the 1-(pyrrol-2-yl) eudistomin group, which we have named neoeudistomin 1.

Chart III

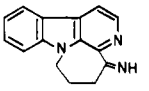


Neoeudistomin 2 (**19**, Scheme IV), with a 6-bromo substituent, was prepared from the key intermediate **43** (obtained by treatment of **41** with bromine in THF) by similar reaction with the Grignard reagent to give **46**, which was cyclized.

Repetition of the reaction sequence, starting with 5-methoxytryptamine, gave the intermediate 1-cyano-6-methoxy-β-carboline (**42**, Scheme III). The Grignard reaction and cyclization yielded *O*-methyleudistomin M (**47**, Scheme IV), which was demethylated with boron tribromide to provide eudistomin M.[4]

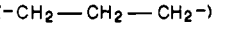
The structure of eudistomin B has not been completely assigned. Signals for a 1,6,7-trisubstituted β-carboline nucleus and a methoxyl group appear in its ^{1}H NMR spectrum.[4]

Eudistomins G, H, I, P, and Q. The toluene-soluble layer from *E. olivaceum* yielded eudistomins G, H, and I, whose structures were assigned as **7–9**, respectively, on the basis of UV,[4] IR, and NMR[4] spectroscopy and FABMS.[1b] The presence of an imino carbon, suggested by ^{13}C NMR signals,[1b] was supported by reduction of eudistomin G to dihydroeudistomin G (**24**, Chart II), followed by acetylation to acetyldihydroeudistomin G (**25**, Chart II) and comparison of UV data to those for the β-carboline harman.[1b] Two ways to attach the $-CH_2CH_2CH_2-$ (C-3′, C-4′, C-5′) unit were considered: (1) to the indole nitrogen to give an iminoazepine (e.g., **9′**) or (2) to the imino nitrogen to give a

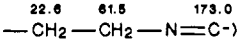


9′

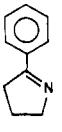
pyrroline ring. In agreement with the 1-pyrrolin-2-yl structure, the EIMS fragmentation [*m/z* 235 (M), M − H, M − 27, M − 28, M − 41, M − 42, M − 68, and *m/z* 68] of eudistomin I (**9**) is very similar to that of 2-(2-pyrrolinyl)pyridine (apoferrosamine),[10] in which almost all fragment ions derive from the pyrrolinyl unit. The ^{13}C chemical shifts of the three-carbon unit



and the imino carbon (176.7, N=C) of **9** also argue that the structure contains a 1-pyrrolinyl ring, since they match closely those of the pyrroline moiety



of 2-phenyl-1-pyrroline (**48**),[11] synthesized as a model compound.



48

Further evidence was obtained from strenuous acetylation of acetyldihydroeudistomin G (**25**) to give the diacetyl derivative **26** (Chart II); a diacetyl derivative of **9′** would not be expected.

(1) Preliminary communications of portions of the present work: (a) Rinehart, K. L., Jr.; Kobayashi, J.; Harbour, G. C.; Hughes, R. G., Jr.; Mizsak, S. A.; Scahill, T. A. *J. Am. Chem. Soc.* **1984**, *106*, 1524–1526. (b) Kobayashi, J.; Harbour, G. C.; Gilmore, J.; Rinehart, K. L., Jr. *Ibid.* **1984**, *106*, 1526–1528. (c) Taken in part from: Harbour, G. C. Ph.D. Dissertation, University of Illinois at Urbana—Champaign, Urbana, IL, 1983. (d) Taken in part from: Mascal, M. J. M.Sc. Dissertation, University of Illinois at Urbana—Champaign, Urbana, IL, 1986. (e) Presented in part at the 187th ACS National Meeting, St. Louis, MO, April 8–13, 1984, Paper ORGN 70, and at the 1984 International Chemical Congress of the Pacific Basin Societies, Honolulu, HI, December 16–21, 1984, Paper 10E48.

(2) (a) University of Illinois. (b) Laboratoire Arago.

(3) Rinehart, K. L., Jr.; Shaw, P. D.; Shield, L. S.; Gloer, J. B.; Harbour, G. C.; Koker, M. E. S.; Samain, D.; Schwartz, R. E.; Tymiak, A. A.; Weller, D. L.; Carter, G. T.; Munro, M. H. G.; Hughes, R. G., Jr.; Renis, H. E.; Swynenberg, E. B.; Stringfellow, D. A.; Vavra, J. J.; Coats, J. H.; Zurenko, G. E.; Kuentzel, S. L.; Li, L. H.; Bakus, G. J.; Brusca, R. C.; Craft, L. L.; Young, D. N.; Connor, J. L. *Pure Appl. Chem.* **1981**, *53*, 795–817.

(4) See supplementary material.

(5) Ho, B. T.; McIsaac, W. M.; Walker, K. E.; Estevez, V. *J. Pharm. Sci.* **1968**, *57*, 269–274.

(6) Ho, B. T.; Li, K.-C.; Walker, K. E.; Tansey, L. W.; Kralik, P. M.; McIsaac, W. M. *J. Pharm. Sci.* **1970**, *59*, 1445–1448.

(7) Fu, P. P.; Harvey, R. G. *Tetrahedron Lett.* **1974**, 3217–3220.

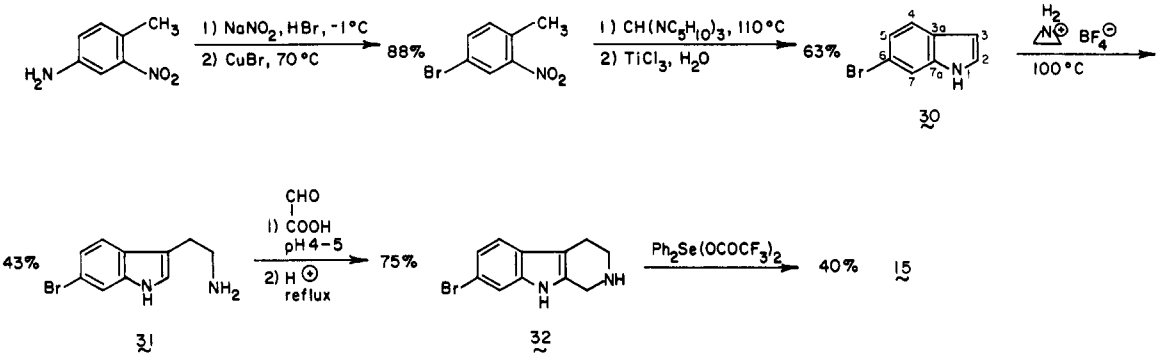
(8) Stowell, J. C. *J. Org. Chem.* **1976**, *41*, 560–561.

(9) Elming, N.; Clauson-Kass, N. *Acta Chem. Scand.* **1952**, *6*, 867–874.

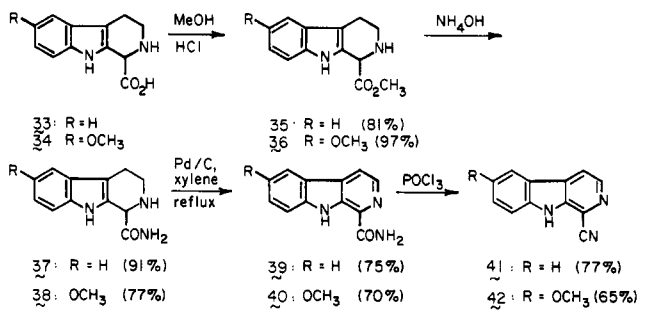
(10) Thouvenot, M. P.; Gaudemer, A.; Barbier, M. *Bull. Soc. Chim. Biol.* **1965**, *47*, 2085–2094.

(11) Starr, D. F.; Bulbrook, H.; Hixon, R. M. *J. Am. Chem. Soc.* **1932**, *54*, 3971–3976.

Scheme II



Scheme III



The poor yield (~10%) of **26** is presumably due to steric hindrance of the pyrrolidinyl ring around the indole NH group and the lower basicity of the indole NH. As final confirmation of the 1-pyrrolin-2-yl structure, eudistomins H and I were synthesized as described below.

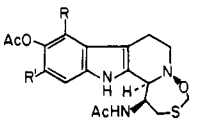
Eudistomins P and Q, more polar compounds with the pyrrolinyl-β-carboline system, were isolated as minor products from the chloroform layer that yielded eudistomins A–F and J–O.[4] They were assigned structures **16** and **17**, and eudistomin Q was synthesized as described next.

Syntheses of 1-(1-pyrrolin-2-yl) eudistomins (H, I, and Q) required the building of a dihydropyrrole ring by an adaptation of the route used for synthesis of pyrrolyl-substituted eudistomins. This involved in situ sodium borohydride reduction of the intermediate imine formed by reaction of a Grignard reagent with a nitrile (Schemes IV and V). Since the required isomerization of **52** to **9** could not be achieved effectively in one step by acid or base or noble metal catalysis, **52** was reduced[12] to the 1-(pyrrolidin-2-yl) **55** (Scheme V), which was then oxidized to eudistomin I (**9**) by N-chlorination followed by dehydrohalogenation.

Eudistomins H and Q were obtained from parallel sequences (Scheme V), and the ^{1}H NMR spectral patterns of synthetic **8**, **9**, and **17** matched those of the natural products.

Synthesis of a Model Ring System. When both the pyrrolinyl (e.g., **7–9**) and iminoazepine (e.g., **9′**) structures were under consideration, the acetyl derivative (**59**, Chart III) of the compound which would be expected as the borohydride reduction product of the iminoazepino-β-carboline **9′** was synthesized. The 4-substituted 3,7a-diazacyclohepta[*jk*]fluorene ring system was synthesized via a 1,9-disubstituted β-carboline, employing a Dieckmann cyclization in the key step (Scheme VI), and then compared with the product (**25**) from reduction and acetylation of eudistomin G. The proton resonances of the azacycloheptene ring were assigned by proton decoupling experiments. The ^{1}H NMR chemical shifts of the three-methylene unit ($-CH_2-C-H_2-CH_2-$) of **25** differed considerably from those of **59**, as shown in Chart III, confirming structures **7–9**.

Eudistomins C, E, F, K, and L, another group of eudistomins isolated from the chloroform layer of *E. olivaceum*, contain an oxathiazepine ring attached to a tetrahydro-β-carboline nucleus and were assigned structures **3**, **5**, **6**, **11**, and **12**, respectively,[1a] on the basis of spectral data for the pure compounds as well as for diacetyleudistomins C (**64**) and E (**65**).



	R	R′
64 (C – Ac_2)	H	Br
65 (E – Ac_2)	Br	H

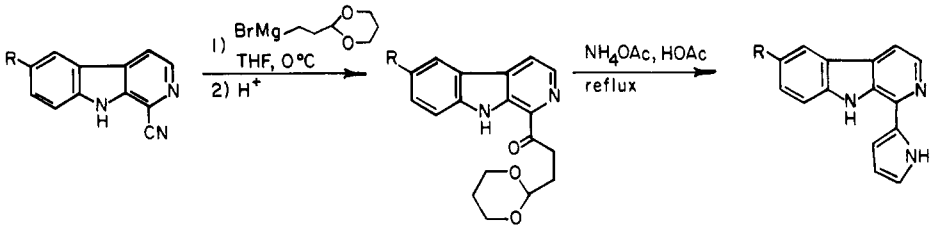
Antiviral Activity. Four groups of eudistomins have been isolated from *E. olivaceum*, including simple β-carbolines (group 1: D, J, N, and O), pyrrolyl-β-carbolines (group 2: A, B, and M), pyrrolinyl-β-carbolines (group 3: G, H, I, P, and Q), and tetrahydro-β-carbolines containing an oxathiazepine ring (group 4: C, E, F, K, and L). The initial activity observed for *Eudistoma olivaceum* extracts was antiviral vs. *Herpes simplex*, type 1 (HSV-1).[1a] The isolated eudistomins were assayed against HSV-1 and showed antiviral activity, but to a widely varying degree (Table I). The most active by far are those containing the oxathiazepine ring (C, E, K, L), and among these C and E, with a phenolic hydroxyl, are active down to 5–10 ng/disk. Eudistomins D, H, K, L, N(+O), and P showed modest inhibition (+) of HSV-1 at 100 ng (L), 250 ng (K), and 500 ng [D, H, N(+O), and P] per 12.5-mm disk. The trend of antiviral potency may be expressed as group 4 (C, E, K, and L) ≫ group 3 (H and P) = group 1 [D and N(+O)] > group 2 (A and B). Besides the substituents on the pyridine ring of the β-carboline, the substituents (Br and/or OH) and their positions on the benzenoid ring of the β-carboline may influence the antiviral activity of eudistomins; the order of antiviral activity observed was E (5-Br, 6-OH) > C (6-OH, 7-Br) > L (6-Br) = K (7-Br) and P (6-OH, 7-Br) = H (6-Br) > G (7-Br) ≈ Q (6-OH) ≈ I (no substitution). The potent antiviral eudistomins C and E were active against RNA viruses (Coxsackie A-21 virus and equine rhinovirus) as well as DNA viruses (HSV-1, HSV-2, and *Vaccinia* virus). Acetylation of the phenol and primary amine functions of eudistomin C effects a 100-fold reduction in activity.

Antimicrobial Activity. A Remarkable Case of Synergism. The initial extract of *E. olivaceum* showed activity vs. *Bacillus subtilis* on shipboard and samples collected later showed on-site activity vs. *B. subtilis*, *Escherichia coli*, *Saccharomyces cerevisiae*, and *Penicillium atrovenetum*. Here again, the isolated eudistomins are also antimicrobial to a widely differing degree, with the oxathiazepines being generally the most active (Table I). In this case, oxathiazepino-eudistomins with (C and E) and without (K and L) a phenolic hydroxyl are equally active. Some eudistomins lacking an oxathiazepine ring are also antimicrobial.

Some eudistomins are active against *B. subtilis* (D, I, and Q) or *S. cerevisiae* (H) or both (P), while others are active against *E. coli* and *P. atrovenetum* [C, K, L, and N(O)] in addition to *B. subtilis* and *S. cerevisiae* (Table I). Where the bioactivities

(12) Billman, J. H.; McDowell, J. W. *J. Org. Chem.* **1961**, *26*, 1437.

Scheme IV

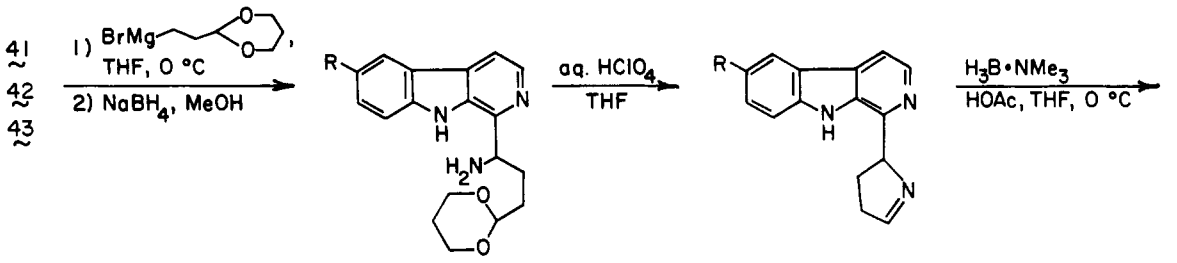


41 R = H
42 : R = OCH_3
43 : R = Br

44 : R = H (84%)
45 : R = OCH_3 (75%)
46 : R = Br (74%)

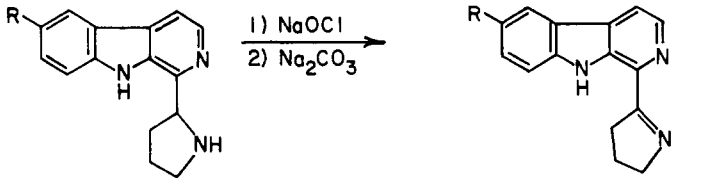
18 : R = H (88%)
47 : R = OCH_3 (67%)
19 : R = Br (92%)

Scheme V



49 : R = H (78%)
50 : R = OCH_3 (68%)
51 : R = Br (79%)

52 : R = H (74%)
53 : R = OCH_3 (68%)
54 : R = Br (50%)



55 : R = H (75%)
56 : R = OCH_3 (77%)
57 : R = Br (62%)

9 : R = H (75%)
58 : R = OCH_3 (74%)
8 : R = Br (80%)

of the synthetic eudistomins were tested, they matched those of the natural products. For example, synthetic eudistomin D was antiviral at 500 ng/disk and somewhat antibacterial vs. *Bacillus subtilis* at 100 μg/disk.

The most interesting activity is that of a mixture of eudistomins N and O, which displays a remarkable degree of synergism. The mixture of N and O originally isolated was quite antimicrobial (Table I), but eudistomin N, the first of the two synthesized, was essentially inactive at 100 μg/disk. We speculated that the original activity must have been due to eudistomin O, but synthetic eudistomin O also proved inactive. The presumption that a trace impurity in the natural mixture caused the activity was shown to be incorrect when a mixture of synthetic eudistomins N and O displayed antimicrobial activity like that of the natural mixture.

Calcium Release from Sarcoplasmic Reticulum. Work carried out at the Mitsubishi-Kasei Institute of Life Sciences has demonstrated that several of the eudistomins induce calcium release from fragmented sarcoplasmic reticulum.[13] The effect is especially pronounced with 7-bromoeudistomin D (**66**, Chart I), obtained by demethylation of 7-bromo-*O*-methyleudistomin D, a side reaction product from bromination of 6-methoxy-β-carboline (**28**; cf. Experimental Section). The calcium-releasing effect of **66** is 400 times more potent than that of caffeine.[14]

Biosynthetic Considerations. All the eudistomins may be derived biosynthetically from 1 mol of tryptophan (C-3–C-9a, N-2, N-9). Eudistomins A and M, as well as G, H, I, P, and Q, are presumed to contain, in addition, glutamate-derived units—C-1 and the pyrrole ring in A and M, C-1 and the pyrrolinyl ring in G, H, I, P, and Q. On the other hand, eudistomins C, E, K, and L can be considered to be derived from tryptophan (N-2–C-9a) and cysteine (C-1, C-10, C-11, and S-12).

The eudistomin content of *E. olivaceum* appears to depend on the site of collection. For example, eudistomins K and L were isolated from *E. olivaceum* collected in Florida (IFE 21-V-82-1-3) but not from that collected in Belize (IRCE 1-VII-81-3-1). A detailed study of eudistomin content with respect to the ecology of *E. olivaceum* is required to reveal the effects of the time and place of collection and the age of the tunicate.

Experimental Section

General. See supplementary material.

Tunicate Collection. *Eudistoma olivaceum* samples were collected by snorkeling and by wading, usually among the roots of mangroves, with

(13) Kobayashi, J.; Ohizumi, Y.; Gilmore, J.; Rinehart, K. L., Jr. 15th IUPAC International Symposium on the Chemistry of Natural Products, The Hague, Netherlands, Aug. 17–22, 1986; Abstracts.

(14) Nakamura, Y.; Kobayashi, J.; Gilmore, J.; Mascal, M.; Rinehart, K. L., Jr.; Nakamura, H.; Ohizumi, Y. *J. Biol. Chem.* **1986**, *261*, 4139–4142.

Scheme VI

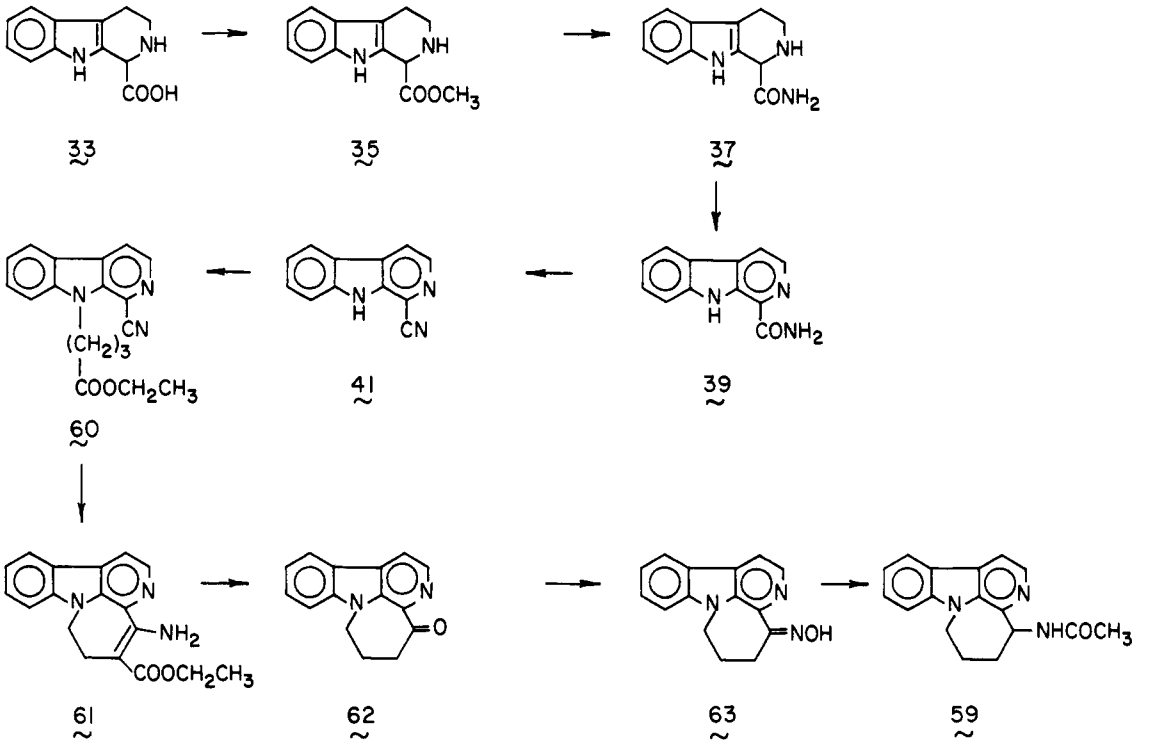


Table I. Antiviral and Antimicrobial Activity of the Eudistomins and Derivatives

eudistomin	HSV-1 assay[b] (ng/12.7-mm disk)	antimicrobial assay[a] *Bacillus subtilis*	*Escherichia coli*	*Saccharomyces cerevisiae*	*Penicillium atrovenetum*
4 (D)	+(500)	14 (clear)	0	0	0
4 (D synth.[c])	+(500)	17 (faint)	0	0	0
22 (D-Ac_2)	+(500)				
22 (D-Ac_2 synth.[c])	±(1250)	0	0	0	0
66 (7-Br-D synth.[c])	+(500)	0		0	0
10 (J)	±(1000)	0	0	0	0
20 (J-Ac_2)	±(500)	0	0	0	0
14 + **15** (N + O)	+(500)	19 (clear)	18 (clear)	25 (faint)	20 (clear)
14 (N synth.[c])	–[d]	14 (faint)	0	0	0
15 (O synth.[c])	–[d]	0	0	0	0
14 + **15** (N synth. + O synth. [c])	±[d]	19[e]	16	0	25[f]
1 (A)	–(500)	0	0	0	0
23 (A-Ac)	+(1000)	0	0	0	0
21 (M-Ac)	±(500)	0	0	0	0
2 (B)	–(500)				
7 (G)	±(500)	0	0	0	0
25 (diacetyldihydro G)	±(200)	15 (clear)	0	0	0
8 (H)	+(500)	0	0	20 (faint)	0
9 (I)	±(500)	14 (clear)	0	0	0
16 (P)	+(500)	15 (clear)	0	20 (faint)	0
17 (Q)	±(500)	14 (clear)	0	0	0
3 (C)	+++(50)	26	22	0	27
	++(25)	14[g]	0[g]	0[g]	0[g]
	+(10)	0[h]	0[h]	0[h]	0[h]
	–(5)				
64 (C-Ac_2)	+(1000)	0	0	0	0
5 (E)	+++(50)	17[h]	0[h]	0[h]	0[h]
	+++(25)	0[i]	0[i]	0[i]	0[i]
	±(5)				
11 (K)	+(250)	23 (clear)	15 (clear)	24 (clear)	27 (clear)
12 (L)	+(100)	27 (clear)	20 (clear)	28 (clear)	32 (clear)

[a] Zone of inhibition (mm) for 100 μg/12.7-mm disk, 37 °C, 20 or 16 h. [b] Degree of inhibition (see text): +++ (complete inhibition), ++, +, ±, – (no inhibition). [c] Sample prepared by total synthesis. [d] 10 μg/6.35-mm disk. [e] N synth. and O synth. were inactive but (N synth. + O synth.) showed a 13-mm zone of inhibition, all at 10 μg/6.35-mm disk vs. *Micrococcus luteus*. [f] 50 μg/12.7-mm disk. [g] 5 μg/12.7-mm disk. [h] 1 μg/12.7-mm disk. [i] 0.1 μg/12.7-mm disk.

help from Dr. G. J. Bakus, F. Good, J. Piraino, H. Reichard, J. Marsh, K. L. Rinehart, III, and J. B. Rinehart, and were stored at –20 °C. Sample No. 553, AHCE 16-III-78-1-4 (0.3 kg), was collected at Banco Chinchorro, Mexico (18° 35.2′ N, 87° 20.6′ W) on the west side of Cayo Centro at 1–3-ft depths. IRCE 1-VII-81-3-1 (3.4 kg) was collected at Lighthouse Reef, Belize (17° 29′ N, 88° 10′ W), at its NE corner. IFE 21-V-82-1-3 (0.9 kg) was collected at Island No. 179, Indian River, FL (27° 26.8′ N, 80° 19.6′ W).

Biological Assays. Antimicrobial assays were performed by the method of Shaw et al.[15] Pure compounds were dissolved in methanol or

chloroform (1 mg/mL) for bioassay. The disk diffusion method was used to test for growth inhibition (usually at 100 μL/12.7-mm disk, except as noted) on agar lawns of *Bacillus subtilis*, *Micrococcus luteus*, *Escherichia coli*, *Saccharomyces cerevisiae*, and *Penicillium atrovenetum*. Antiviral activity and cytotoxicity were assayed by using a standard procedure[16] involving monkey kidney cells (CV-1 line). The cytotoxic zone appeared as an unstained area whose diameter is reported in mm (≤36 mm, the well diameter). Antiviral activity as indicated by inhibition of viral plaque formation was assigned qualitatively as complete inhibition (+++), a few plaques around the outside of the well (++), definite inhibition (+), questionable inhibition (±), and no inhibition (−).

Isolation (see scheme in supplementary material). *Eudistoma olivaceum* (IRCE 1-VII-81-3-1, 1.5 kg) was extracted with methanol–toluene (3:1, 3 × 1 L) in a Waring blender. The filtrate was then partitioned with aqueous sodium nitrate (1 N, 1 L) to give an aqueous layer and a toluene layer. The aqueous layer was washed with toluene (2 × 1 L), and the toluene extracts were combined and evaporated to give 7.50 g of a brown oil. The aqueous layer was then extracted with chloroform (3 × 0.5 L), and the chloroform extracts were evaporated to give 1.32 g of a dark green oil. Extraction of the aqueous layer with ethyl acetate (3 × 0.5 L) and evaporation gave 0.78 g of a brown oil. Finally, partitioning the aqueous layer against 1-butanol gave a 1-butanol layer and an aqueous layer. Evaporation of the 1-butanol layer gave 5.85 g of a brown oil. The aqueous layer was lyophilized to give 10.6 g of powder, including sodium nitrate. The antiviral and antimicrobial activities of all extracts were examined.

The toluene-soluble material (7.50 g) was subjected to silica gel column chromatography with chloroform to give a mixture of eudistomins G, H, and I; eudistomin G (**7**, 23 mg, 0.0015% wet weight) crystallized from hexane–ethyl acetate (2:1) and was recrystallized from methylene chloride to yield colorless needles. The mother liquor on C_{18} reversed-phase MPLC (methanol–water, 9:1) gave eudistomin H (**8**, 16 mg, 0.0011%) and eudistomin I (**9**, 15 mg, 0.0010%).

The chloroform-soluble material (1.32 g) was applied to a C_{18} Sep Pak column (methanol) and the eluate was subjected to C_{18} reversed-phase MPLC (methanol–water, 50:50 → 100% methanol) and then to silica gel MPLC (methanol–chloroform, 5:95 → 50:50) and silica gel HPLC to yield eudistomins A (**1**, 16 mg, 0.0011% wet weight, chloroform–methanol, 98:2), B (**2**, 1.1 mg, 0.00008%, 96.5:3.5), C (**3**, 17 mg, 0.0011%, 96.5:3.5), D (**4**, 7.5 mg, 0.0005%, 95:5), a 3:1 mixture of D (**4**) and J (**10**) (9 mg, 0.0006%, 95:5), E (**5**, 18 mg, 0.0012%, 95:5), F (**6**, 10.5 mg, 0.0008%, 93:7), P (**16**, 3.7 mg, 0.0002%, 99:1), and Q (**17**, 0.5 mg, 0.00003%, 96.5:3.5).

After acetylation with pyridine and acetic anhydride, eudistomins J (**10**) and M (**13**) were isolated as their acetyl derivatives (**20**, from J, 0.4 mg, 0.00002%; **21**, from M, 0.7 mg, 0.00004%) by silica gel HPLC with chloroform. Acetyl derivatives of eudistomins D, A, C, and E (**22**, **23**, **64**, **65**) were obtained by acetylation with pyridine and acetic anhydride followed by silica gel HPLC with chloroform.

Similarly, the toluene (5.30 g) and the chloroform-soluble (1.32 g) materials were obtained from 1.1 kg of *E. olivaceum* (IRCE 1-VII-81-3-1). Eudistomins C (**3**, 8 mg, 0.0007%), E (**5**, 5 mg, 0.0005%), and a 3:1 mixture of D (**4**) and J (**10**) (5 mg, 0.0005%) were isolated by the purification procedure described above. Furthermore, eudistomins K (**11**, 6 mg, 0.0007%, 98:2), L (**12**, 4 mg, 0.0004%, 98:2), and a 1:1 mixture of N (**14**) and O (**15**) (2 mg, 0.0002%, 95:5) in addition to eudistomins A (**1**, 7 mg, 0.0008%, 98:2), C (**3**, 3 mg, 0.0003%, 96.5:3.5), and a 3:1 mixture of D (**4**) and J (**10**) (2 mg, 0.0002%, 95:5) were isolated from extracts of *E. olivaceum* (IFE 21-V-82-1-3, 920 g) by the same extraction and isolation techniques. The isolation and purification procedures were monitored at each step by thin-layer chromatography (TLC) and in vitro antimicrobial and antiviral assays on extracts and column fractions.

Eudistomin A (1): yellow oil; UV;[4] IR;[4] ^{1}H and ^{13}C NMR.[4] Anal. Calcd for $C_{15}H_{11}BrN_3O$ (M + H): 328.0085. Found: 328.0067 (HRFABMS).

Acetyleudistomin A (23). Eudistomin A was treated with acetic anhydride and pyridine to give its acetyl derivative (**23**): UV;[4] IR;[4] ^{1}H NMR.[4] Anal. Calcd for $C_{17}H_{13}BrN_3O_2$ (M + H): 370.0209. Found: 370.0189 (HRFABMS).

Eudistomin B (2): light yellow solid; UV;[4] ^{1}H NMR.[4] The compound shows an M + H ion by FABMS at *m/z* 374, with a bromine isotope peak at *m/z* 376.

Eudistomin C (3): pale yellow oil; $[\alpha]^{25}_D$ −52° (*c* 0.4, MeOH); UV;[4] IR;[4] ^{1}H NMR.[4] Anal. Calcd for $C_{14}H_{17}BrN_3O_2S$ (M + H): 370.0147. Found: 370.0173 (HRFABMS).

Diacetyleudistomin C (64). Eudistomin C was treated with acetic anhydride and pyridine to give its diacetyl derivative (**64**): $[\alpha]^{25}_D$ −43° (*c* 0.8, $CHCl_3$); UV;[4] IR;[4] ^{1}H NMR.[4] Anal. Calcd for $C_{18}H_{20}BrN_3O_4S$: mol wt, 453.0358. Found: mol wt, 453.0350 (HREIMS).

Eudistomin D (4): yellow oil; UV;[4] IR;[4] ^{1}H NMR.[4] Anal. Calcd for $C_{11}H_8BrN_2O$ (M + H): 262.9819. Found: 262.9830 (HRFABMS).

Diacetyleudistomin D (22). Eudistomin D was treated with acetic anhydride in pyridine at room temperature overnight to give its diacetyl derivative (**22**) as a yellow oil: UV;[4] IR;[4] ^{1}H NMR.[4] Anal. Calcd for $C_{15}H_{12}BrN_2O_3$ (M + H): 347.0031. Found: 347.0038 (HRFABMS).

Eudistomin E (5): pale yellow oil; $[\alpha]^{25}_D$ −18° (*c* 0.1, MeOH); UV;[4] IR;[4] ^{1}H NMR.[4] Anal. Calcd for $C_{14}H_{17}BrN_3O_2S$ (M + H): 370.0147. Found: 370.0165 (HRFABMS).

Diacetyleudistomin E (65). Eudistomin E was treated with acetic anhydride and pyridine to give its diacetyl derivative (**65**): $[\alpha]^{25}_D$ +18° (*c* 0.5, $CHCl_3$); UV;[4] IR, same as **64**;[4] ^{1}H and ^{13}C NMR.[4]

The molecular formula and fragmentation patterns were established by HREIMS: M, $C_{18}H_{20}BrN_3O_4S$, calcd 453.0358 (found 453.0354); M − C_4H_7NO, $C_{14}H_{13}BrN_2O_3S$, 367.9831 (367.9826); *m/z* 368 − CH_2S, $C_{13}H_{11}BrN_2O_3$, 321.9953 (321.9942); *m/z* 322 − C_2H_2O, $C_{11}H_9BrN_2O_2$, 279.9847 (279.9843); *m/z* 280 − OH, $C_{11}H_8BrN_2O$, 262.9820 (262.9812).

CD Spectra of Diacetyleudistomins C (64) and E (65). The CD curves[4] for the diacetyl derivatives of eudistomins C and E were obtained at 21.6 μg/mL (**64**) and 24.0 μg/mL (**65**) in methanol at room temperature with use of a 1-cm quartz cell.

Eudistomin F (6): light yellow solid; UV;[4] IR;[4] ^{1}H NMR.[4] FABMS shows pseudomolecular ions at *m/z* 428 (M + H) and 426 (M - H) in the positive and negative ion spectra, respectively. The molecular formula and fragmentation patterns were established by HREIMS: M, $C_{16}H_{18}BrN_3O_4S$, calcd 427.0200 (found 427.0202); M - $C_4H_7NO_2$, $C_{12}H_{11}BrN_2O_2S$, 325.9724 (325.9723); *m/z* 326 − CH_2S, $C_{11}H_9BrN_2O_2$, 279.9847 (279.9851); *m/z* 280 − OH, $C_{11}H_8BrN_2O$, 262.9819 (262.9819).

Eudistomin G (7): colorless needles; mp 204–206 °C; UV;[4] IR;[4] ^{1}H and ^{13}C NMR.[4] Anal. Calcd for $C_{15}H_{12}BrN_3$: mol wt, 313.0215. Found: mol wt, 313.0195 (HREIMS).

Dihydroeudistomin G (24). Eudistomin G was reduced with sodium borohydride in methanol at reflux for 1 h to amine **24**: UV;[4] FABMS *m/z* 316 and 318 (M + H; ^{79}Br, ^{81}Br).

Acetyldihydroeudistomin G (25). Acetylation of **24** with acetic anhydride in pyridine at room temperature gave the monoacetyl derivative **25**: UV;[4] IR;[4] ^{1}H NMR.[4] Anal. Calcd for $C_{17}H_{17}BrN_3O$ (M + H): 358.0555. Found: 358.0558 (HRFABMS).

Diacetyldihydroeudistomin G (26). Treatment of **25** with acetic anhydride and 4-(dimethylamino)pyridine in pyridine at 60 °C for 2 days gave the diacetyl derivative **26**, which was purified by silica gel HPLC with chloroform to a yellow oil: IR.[4] Anal. Calcd for $C_{19}H_{19}BrN_3O_2$ (M + H): 400.0661. Found: 400.0685 (HRFABMS).

Eudistomin H (8): yellow powder; mp 140–142 °C; UV;[4] ^{1}H and ^{13}C NMR.[4] Anal. Calcd for $C_{15}H_{12}BrN_3$: mol wt, 313.0215. Found: mol wt, 313.0208 (HREIMS).

Eudistomin I (9): colorless powder; mp 153–155 °C; UV;[4] IR;[4] ^{1}H and ^{13}C NMR.[4] Anal. Calcd for $C_{15}H_{13}N_3$: mol wt, 235.1109. Found: mol wt, 235.1094 (HREIMS).

Eudistomin J (10) has not been isolated. The ^{1}H NMR spectrum of a 1:1 mixture of eudistomins D and J was obtained, however, and all the signals of eudistomin J were clearly assigned by subtracting the ^{1}H NMR spectrum of eudistomin D.[4] A mixture containing eudistomin D as the major component and eudistomin J as the minor component was treated with acetic anhydride in pyridine at room temperature overnight and then was subjected to silica gel HPLC with $CHCl_3$ to give diacetyleudistomin D (**22**, described above) and diacetyleudistomin J (**20**).

Diacetyleudistomin J (20): yellow oil; UV;[4] IR;[4] ^{1}H NMR.[4] Anal. Calcd for $C_{15}H_{12}BrN_2O_3$ (M + H): 347.0031. Found: 347.0029 (HRFABMS).

Eudistomin K (11): slightly yellow oil; $[\alpha]^{25}_D$ −102° (*c* 0.2, MeOH); UV;[4] IR;[4] ^{1}H NMR.[4] Anal. Calcd for $C_{14}H_{17}BrN_3OS$ (M + H): 354.0276. Found: 354.0272 (HRFABMS).

Eudistomin L (12): slightly yellow oil; $[\alpha]^{25}_D$ −77° (*c* 0.2, MeOH); UV;[4] IR;[4] ^{1}H NMR.[4] Anal. Calcd for $C_{14}H_{17}BrN_3OS$ (M + H): 354.0276. Found: 354.0282 (HRFABMS).

Eudistomin M (13) has not been isolated from the tunicate. A mixture containing eudistomin C as the major component and eudistomin M as the minor component was treated with acetic anhydride in pyridine at room temperature overnight and then subjected to silica gel HPLC with chloroform to give diacetyleudistomin C (**64**, described above) and acetyleudistomin M (**21**) as a yellow oil: UV;[4] IR;[4] ^{1}H NMR.[4] Anal. Calcd

(15) Shaw, P. D.; McClure, W. O.; Van Blaricom, G.; Sims, J.; Fenical, W.; Rude, J. *Food and Drugs from the Sea 1974*; Webber, H. H., Ruggieri, G. D., Eds.; Marine Technological Society: Washington, DC, 1976; pp 429–433.

(16) Schroeder, A. C.; Hughes, R. G., Jr.; Bloch, A. *J. Med. Chem.* **1981**, *24*, 1078–1083.

for $C_{17}H_{14}N_3O_2$ (M + H): 292.1095. Found: 292.1086 (HRFABMS).

Eudistomin N (14) and eudistomin O (15) were isolated as a 1:1 mixture: yellow oil; UV;[4] IR;[4] ^{1}H NMR.[4] Anal. Calcd for $C_{11}H_8BrN_2$ (M + H): 246.9868. Found: 246.9871 (HRFABMS).

Eudistomin P (16): yellow powder; mp 128–130 °C; UV;[4] IR;[4] ^{1}H NMR.[4] Anal. Calcd for $C_{15}H_{13}BrN_3O$ (M + H): 330.0258. Found: 330.0242 (HRFABMS).

Eudistomin Q (17): yellow powder; mp 120–125 °C; UV;[4] IR[4]; ^{1}H NMR.[4] Anal. Calcd for $C_{15}H_{14}N_3O$ (M + H): 252.1134. Found: 252.1137 (HRFABMS).

6-Bromo-β-carboline (Eudistomin N, 14). Bromine (0.476 g, 5.96 mmol) was added to β-carboline (**27**, 0.50 g, 2.98 mmol) in tetrahydrofuran (THF, 50 mL). The reaction mixture was stirred at room temperature for 1 h, shaken with aqueous sodium thiosulfate, made basic with concentrated ammonia, and extracted with chloroform. Workup and recrystallization from methanol/chloroform gave 0.574 g (78%) of **14** as yellow needles: mp 265–268 °C dec; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{11}H_7{}^{79}BrN_2$: mol wt, 245.9797. Found: mol wt, 245.9795 (HREIMS).

5-Bromo-6-methoxy-β-carboline (29). Bromine (0.202 g, 1.26 mmol) in acetic acid (5 mL) was added to 6-methoxy-β-carboline (**28**, 0.25 g, 1.26 mmol)[5,6] in 100 mL of acetic acid. The reaction mixture was stirred overnight at room temperature, and then solvent was removed in vacuo and the crude product was extracted from aqueous sodium bicarbonate with chloroform. Workup and recrystallization from xylene gave 0.25 g (71%) of **29** as yellow needles: mp 218–219 °C; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{12}H_9{}^{79}BrN_2O$: mol wt, 275.9900. Found: mol wt, 275.9899 (HREIMS).

5-Bromo-6-hydroxy-β-carboline (Eudistomin D, 4). Boron tribromide (**33**%) in dichloroethane (10 mL) was added to **29** (0.25 g, 0.90 mmol) in dichloroethane (100 mL). The solution was heated at reflux for 0.5 h and then cooled, and the excess reagent was destroyed by slow addition of methanol. The reaction mixture was evaporated to dryness in vacuo, made basic with concentrated ammonia, and reevaporated. The crude product was purified by flash chromatography on silica, eluting with methanol/chloroform (7.5:92.5), to give 0.214 g (90%) of **4** as a yellow amorphous solid: mp >280 °C; UV, IR, ^{1}H NMR.[4] Anal. Calcd for $C_{11}H_7{}^{79}BrNO$: mol wt, 261.9748. Found: mol wt, 261.9745 (HREIMS).

7-Bromoeudistomin D (66). Boron tribromide (33%) in dichloroethane (5 mL) was added to the crude bromination product from **28** (50 mg, obtained as above) in dichloroethane (50 mL). The solution was heated at reflux for 20 min and then cooled, and the excess reagent was destroyed by slow addition of methanol. The reaction mixture was evaporated to dryness in vacuo, made basic with concentrated ammonia, and reevaporated. The crude product was purified by flash chromatography on silica, eluting with methanol/chloroform (5:95) to give **66** (9.5 mg) [^{1}H NMR (CD_3OD) δ 8.90 (br s, 1), 8.71 (d, 1, J = 5.0 Hz), 8.33 (br d, 1, J = 5.0 Hz), 7.39 (s, 1)] followed by **4** (24.3 mg).

4-Bromo-2-nitrotoluene. A solution of sodium nitrite (4.39 g, 63.6 mmol) in water (7.5 mL) was added slowly to a suspension of 4-amino-2-nitrotoluene (9.33 g, 61.3 mmol) in hydrobromic acid (16%, 200 mL) at −1 °C. The resulting diazo solution was suction filtered into a solution of freshly prepared[17] copper(I) bromide (10.0 g, 69.7 mL), and the mixture was stirred at 70 °C for ca. 1 h and then extracted with ether. The ethereal extract was washed with dilute potassium hydroxide, concentrated hydrobromic acid, and water. Evaporation and column chromatography (silica, 2–8% EtOAc in C_6H_{12}) yielded 11.58 g (88%) of the title compound as a very pale yellow crystalline mass: mp 45–46 °C (from EtOH/H_2O, lit.[18] mp 47 °C); ^{1}H NMR.[4]

6-Bromoindole (30). A mixture of 4-bromo-2-nitrotoluene (3.96 g, 18.3 mmol) and tripiperidinomethane (7.30 g, 27.5 mmol) was stirred at 110 °C under water aspiration for ca. 4 h while piperidine was removed and (*E*)-4-bromo-2-nitro-β-piperidinostyrene formed. The reaction mixture was taken up in a minimum volume of acetone and introduced into a separatory funnel containing titanium(III) chloride (20% aqueous solution, J. T. Baker Chemical Co., 87.9 g, 114 mmol) and 4 M ammonium acetate buffer (170 mL). The mixture was shaken for 10 min and then extracted with ether (4×). Evaporation and column chromatography (silica, benzene) yielded 2.25 g (63%) of product which coeluted with a deep-red impurity which could not be removed by treatment with Norit or by further chromatography. Sublimation at 70 °C (0.01 Torr), however, afforded white crystals of **30**: mp 95–96 °C (lit.[19] 94 °C); UV, IR, ^{1}H and ^{13}C NMR, EIMS.[4] Anal. (C_8H_6BrN) C, N, H.

6-Bromotryptamine (31). A. Aminoethylation. Aziridinium tetrafluoroborate (0.660 g, 5.04 mmol)[20,21] was added in portions to **30** (0.986 g, 5.03 mmol) at 100 °C and after 4 h the pasty reaction mixture was quenched with water (50 mL). The base was then liberated by the addition of potassium hydroxide (1 g) and extracted into several volumes of ethyl acetate. Column chromatography (silica, 0–25% CH_3OH and 0–1% concentrated aqueous NH_3 in $CHCl_3$) yielded 0.360 g of **30** and 0.330 g of **31** (43% based on recovery of **30**) as a resinous brown solid: mp 215–217 °C dec (hydrochloride); UV, IR, ^{1}H and ^{13}C NMR, EIMS.[4] Anal. Calcd for $C_{10}H_{11}BrN_2$·HCl: C, 43.59; H, 4.39; N, 10.17. Found: C, 43.47; H, 4.49; N, 9.97.

B. Cyclization. A mixture of 3-bromophenylhydrazine (3.80 g, 20.3 mmol), 4-aminobutanal diethyl acetal (90%, 4.07 mL, 3.80 g, 21.2 mmol), and freshly fused zinc chloride (3.10 g, 22.7 mmol) was heated with stirring in an open flask so that the bath temperature reached 140 °C after 0.5 h. As soon as the mixture was stirred it became quite warm, and during heating in the bath between 60 and 80 °C a steady evolution of ethanol was observed. At 120 °C the mixture was too viscous to stir, but stirring was resumed at 130 °C with the onset of ammonia evolution. When ammonia production ceased, the temperature was raised to and held at 180 °C for 0.5 h. The resulting dark-red filter cake was triturated with 50% acetic acid, and the triturate was basified with sodium hydroxide. Chromatography of the resulting red gum (silica, 10% CH_3OH and 0–1% concentrated aqueous NH_3 in $CHCl_3$) yielded 2.90 g (60%) of product, which was shown by NMR to be a mixture of the 4-bromo and 6-bromo (**31**) isomers, inseparable by silica gel, C-18 reversed-phase, or cyanopropyl HPLC. A clean HPLC separation was effected, however, on an Astec Cyclobond I (β-cyclodextrin) column (10 × 250 mm), eluting with water:methanol:triethylamine:acetic acid (900:100:3:3) to give the 6-bromo and 4-bromo isomers (ca. 1.5:1 ratio by trace integration), differentiated by their NMR aromatic splitting patterns. 4-Bromotryptamine had ^{1}H NMR (Me_2SO-d_6) δ 7.36 (d, 1, J = 8.0 Hz), 7.24 (br s, 1, H-2), 7.14 (d, 1, J = 7.5 Hz), 6.95 (t, 1, J = 7.8 Hz, H-6), 2.98 (t, 2, J = 6.9 Hz, CH_2), 2.85 (br, 2, CH_2).

6-Bromoindole-3-glyoxylamide. Oxalyl chloride (1.34 mL, 1.95 g, 15.4 mmol) was added to a solution of **30** (1.76 g, 8.98 mmol) in anhydrous ether (35 mL) at −2 °C at a rate that did not allow the temperature to exceed 0 °C. After the addition was complete, the mixture was stirred for an additional 30 min at 0 °C and the precipitated 6-bromoindole-3-glyoxylyl chloride was collected on a filter, washed with anhydrous ether, and sucked dry. The bright yellow acid chloride was introduced in small portions with shaking into concentrated aqueous ammonia (30 mL) and stirred at 50 °C for 30 min. The crude amide was obtained as a tan solid which was chromatographed (silica, 10–50% acetone in $CHCl_3$), yielding 0.813 g (34%) of the title compound as a bright yellow crystalline powder: mp 263–264 °C dec; UV, IR, ^{1}H NMR, FIMS.[4] Anal. Calcd for $C_{10}H_7BrN_2O_2$: C, 44.97; H, 2.64; N, 10.49; mol wt, 265.9691. Found: C, 44.96; H, 2.76; N, 10.40; mol wt, 265.9699 (HREIMS).

Attempted reduction of 6-bromoindole-3-glyoxylamide to 6-bromotryptamine (**31**) with lithium aluminum hydride gave a mixture of largely debrominated products.

7-Bromo-1,2,3,4-tetrahydro-β-carboline (32). A solution of glyoxylic acid monohydrate (52.0 mg, 0.565 mmol) in water (0.5 mL) was added dropwise with shaking to a solution of 6-bromotryptamine (**31**) hydrochloride (145 mg, 0.525 mmol) in water (10 mL), then a solution of potassium hydroxide (28.5 mg, 0.508 mmol) in water (0.5 mL) was added, and the mixture was stirred for 1 h at room temperature. The precipitated 7-bromo-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid was removed by filtration and washed with water (1 mL). The damp, light-yellow filter cake was suspended in water (5 mL) and concentrated hydrochloric acid was added slowly in two 0.2-mL portions, the first followed by 30 min at reflux and the second by 15 min. During cooling of the solution the tetrahydro-β-carboline hydrochloride was observed as a dark-brown precipitate, which was immediately replaced, upon adjustment of the pH to ca. 12 with 20% aqueous potassium hydroxide, by a tan precipitate. Collection by filtration and washing with water provided 98.2 mg (75%) of product showing one spot by TLC, which sublimed at 160 °C (0.01 Torr) to give white crystals of **32**: mp 189–190 °C dec; UV, IR, ^{1}H and ^{13}C NMR, EIMS.[4] Anal. Calcd for $C_{11}H_{11}BrN_2$: mol wt, 250.0106. Found: mol wt, 250.0106 (HREIMS).

7-Bromo-β-carboline (Eudistomin O, 15). A. Diphenylselenium Bis(trifluoroacetate) Oxidation. Trifluoroacetic anhydride (0.440 mL, 0.505 g, 2.40 mmol, freshly distilled from phosphorus pentoxide) was added by syringe, with stirring at room temperature during 15 min, to a solution of diphenyl selenoxide (0.599 g, 2.40 mmol, dried for 48 h at 70 °C and 0.01 Torr) in dimethoxyethane (25 mL, distilled from LiAlH). The

(17) Keller, R. N.; Wycoff, H. D. *Inorg. Synth.* **1946**, *2*, 1–4.
(18) *Handbook of Chemistry and Physics*, 60th ed.; Weast, R. C., Ed.; CRC Press: Cleveland, 1979; p C-522.
(19) Plieninger, H. *Chem. Ber.* **1955**, *88*, 370–376.
(20) Harder, U.; Pfeil, E.; Zenner, K.-F. *Chem. Ber.* **1964**, *97*, 510–519.
(21) Reeves, W. A.; Drake, G. L., Jr.; Hoffpauir, C. L. *J. Am. Chem. Soc.* **1951**, *73*, 3522.

resulting diphenylselenium bis(trifluoroacetate) solution was added dropwise over 2 h to a solution of **32** (0.201 g, 0.802 mmol) in dry dimethoxyethane (25 mL). The mixture was stirred for 19 h, acidified with 6 N hydrochloric acid, extracted with ether, and back-extracted with water. The combined aqueous layer was basified with potassium hydroxide and extracted with chloroform. TLC of the chloroform extract indicated the presence of only diphenyl selenoxide and **15**, and column chromatography (silica, 0–3% CH_3OH in $CHCl_3$) provided 79.2 mg (40%) of pale-yellow crystals of **15**: mp 208–210 °C; UV, IR, ^1H and ^{13}C NMR, EIMS.[4] Anal. Calcd for $C_{11}H_7BrN_2$: mol wt, 245.9793. Found: mol wt, 245.9797 (HREIMS).

B. Triphenylmethyl Trifluoroacetate Oxidation. A solution of **32** (100 mg, 0.398 mmol) and triphenylcarbinol (207 mg, 0.795 mmol) in dry trifluoroacetic acid (5 mL) was heated at reflux for 2 h, and the resulting black reaction mixture was neutralized with 20% potassium hydroxide solution (100 mL) and extracted with chloroform. Evaporation provided a gum which was shown by TLC to be a mixture of the starting material, a product very close in R_f to a β-carboline standard, and a number of closely spaced compounds with R_f values greater than that of β-carboline, shown by NMR to be carbolines with incorporated triphenylmethyl groups. Column chromatography (silica, 0–8% methanol and 0–1% concentrated aqueous NH_3 in $CHCl_3$) provided 59.5 mg of unreacted **32** and 6.4 mg (16% based on recovery of **32**) of **15** in the form of a rusty solid that sublimed at 180 °C (0.01 Torr) to give white crystals of **15**.

1-Carbomethoxy-1,2,3,4-tetrahydro-β-carboline (35) Hydrochloride. A solution of 1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid (**33**, 2 g, 9.26 mmol)[5] in methanol (100 mL) saturated with dry hydrogen chloride was stirred overnight at room temperature. The reaction mixture was evaporated to dryness, taken up in a minimum quantity of hot methanol, and crystallized by addition of toluene to give 1.988 g (81%) of **35** hydrochloride as tan needles: mp 212–214 °C dec; UV, IR, ^1H NMR, EIMS.[4] Anal. Calcd for $C_{13}H_{14}N_2O_2$: mol wt, 230.1055. Found: mol wt, 230.1048 (HREIMS).

1-Carbamoyl-1,2,3,4-tetrahydro-β-carboline (37). A solution of **35** hydrochloride (10 g, 37.5 mmol) in concentrated aqueous ammonia (350 mL) was stirred overnight at room temperature. The precipitate was removed by filtration and dried to give 7.37 g (91%) of **37**, which was used without further purification. Recrystallization from methanol gave an analytical sample of **37** as off-white plates: mp 207–209 °C; UV, IR, ^1H NMR, EIMS.[4] Anal. Calcd for $C_{12}H_{13}N_3O$: mol wt, 215.1058. Found: mol wt, 215.1056 (HREIMS).

1-Carbamoyl-β-carboline (39). A mixture of **37** (2 g, 9.30 mmol) and 5% palladium on charcoal (0.50 g) in xylene (250 mL) was heated at reflux for 4 h. The hot solution was filtered and reduced in volume to 100 mL to give on cooling 1.477 g (75%) of **39** as colorless needles: mp 228–228.5 °C (lit.[22] mp 224–226 °C).

1-Cyano-β-carboline (41). Phosphorus oxychloride (5 mL) was added to a solution of **39** (1 g, 4.74 mmol) in toluene (80 mL), the reaction mixture was heated at reflux overnight, and the product was extracted from aqueous ammonia with chloroform. Workup and recrystallization from toluene/chloroform gave 0.701 g (77%) of **41** as yellow needles: mp 230–232 °C (lit.[23] mp 231–232 °C).

1-[3-(1,3-Dioxa-2-cyclohexyl)propanoyl]-β-carboline (44). A solution of 2-(1,3-dioxa-2-cyclohexyl)ethylmagnesium bromide [5.4 mL, prepared from 2-(1,3-dioxa-2-cyclohexyl)ethyl bromide (5.85 mg) and magnesium turnings (0.97 g) in THF (25 mL)[15]] was added over 10 min to a solution of **41** (0.50 g, 2.59 mmol) in dry THF (30 mL), cooled in an ice–water bath. The reaction mixture was stirred at room temperature for 2 h and then diluted with water (30 mL), acidified, shaken briefly to hydrolyze the intermediate imine, basified with aqueous ammonia, and extracted into chloroform. Workup and recrystallization from ethyl acetate/60–80 °C petroleum ether gave 0.673 g (84%) of **44** as yellow needles: mp 139–140 °C; UV, IR, ^1H NMR, EIMS.[4] Anal. Calcd for $C_{18}H_{18}N_2O_3$: mol wt, 310.1317. Found: mol wt, 310.1322 (HREIMS).

1-(Pyrrol-2-yl)-β-carboline (Neoeudistomin 1, 18). Ammonium acetate (0.50 g) was added to a solution of **44** (0.100 g, 0.32 mmol) in acetic acid (5 mL). The reaction mixture was heated at reflux for 3 h, solvent was evaporated, and the crude oil was extracted from aqueous ammonia with chloroform. Workup and purification by column chromatography (silica gel, $CHCl_3$) gave 0.066 g (88%) of **18** as a yellow microcrystalline solid: mp 192–195 °C; UV, IR, ^1H NMR, EIMS.[4] Anal. Calcd for $C_{15}H_{11}N_3$: mol wt, 233.0953. Found: mol wt, 233.0952 (HREIMS).

1-[1-Amino-3-(1,3-dioxa-2-cyclohexyl)propyl]-β-carboline (49). 2-(1,3-Dioxa-2-cyclohexyl)ethylmagnesium bromide solution (22 mL, prepared as for **44**) was added over 10 min to an ice-cold solution of **41** (2.00 g, 10.4 mmol) in dry THF (75 mL). The reaction mixture was stirred at room temperature for 1 h and then cooled in an ice-water bath. Methanol (75 mL) was added and sodium borohydride (1.0 g) was dissolved over 10 min. The reaction mixture was stirred for an additional 0.5 h and then was extracted with chloroform from water. The crude oil obtained after evaporation was purified by column chromatography (silica gel, 0–3% CH_3OH in $CHCl_3$) to give 2.52 g (78%) of **49** as fawn needles: mp 149–150 °C (from CH_2Cl_2/C_6H_{14}); UV, IR, ^1H NMR, EIMS.[4] Anal. Calcd for $C_{18}H_{21}N_3O_2$: mol wt, 311.1634. Found: mol wt, 311.1635 (HREIMS).

1-(1-Pyrrolin-5-yl)-β-carboline (52). Aqueous perchloric acid (10 mL, 17.5% v/v) was added to a solution of **49** (0.25 g, 0.80 mmol) in THF (2.5 mL) with stirring at room temperature for 3 h. Extraction from aqueous ammonia with chloroform, followed by workup and purification by column chromatography (silica, 0–2% CH_3OH in $CHCl_3$), gave 0.14 g (74%) of **52** as an off-white microcrystalline solid: mp 107–110 °C (from CH_2Cl_2); UV, IR, ^1H NMR, EIMS.[4] Anal. Calcd for $C_{15}H_{13}N_3$: mol wt, 235.1109. Found: mol wt, 235.1104 (HREIMS).

1-(Pyrrolidin-2-yl)-β-carboline (55). Borane–trimethylamine complex (0.050 g, 0.68 mmol) was added over 10 min to a solution of **52** (0.20 g, 0.85 mmol) in THF (8 mL) and acetic acid (8 mL), cooled in an ice-water bath. The reaction mixture was stirred for 1 h and then extracted from aqueous ammonia with chloroform. Workup and purification by column chromatography [silica gel; 0–5% CH_3OH in $CHCl_3$, then 5% methanolic NH_3 (10% v/v) in $CHCl_3$] gave 0.152 g (75%) of **55** which crystallized on standing as colorless needles: mp 153.5–155 °C; UV, IR, ^1H NMR, EIMS.[4] Anal. Calcd for $C_{15}H_{15}N_3$: mol wt, 237.1266. Found: mol wt, 237.1262 (HREIMS).

1-(1-Pyrrolin-2-yl)-β-carboline (Eudistomin I, 9). Aqueous sodium hypochlorite (5.25%, 2.1 mL, Clorox) was added to a solution of **55** (0.35 g, 1.48 mmol) in methanol (35 mL). After the reaction mixture was stirred for 10 min, anhydrous sodium carbonate (0.50 g) was added with stirring during an additional 1 h. The reaction mixture was extracted from water with chloroform, and the combined organic extracts were dried and evaporated. The crude product was purified by column chromatography (silica gel, $CHCl_3$) to give **9** (0.261 g, 75%) as colorless plates; mp 150–150.5 °C (from CH_2Cl_2/C_6H_{14}); UV, IR, ^1H NMR, EIMS.[4] Anal. Calcd for $C_{15}H_{13}N_3$: mol wt, 235.1109. Found: mol wt, 235.1106 (HREIMS).

6-Bromo-1-cyano-β-carboline (43). Bromine (0.25 mL, 2 equiv) was added dropwise to a solution of **41** (0.50 g, 2.59 mmol) in THF (40 mL). The reaction mixture was stirred for 1 h and then extracted from aqueous ammonia with 25% methanol in chloroform. The combined organic extracts were washed with dilute aqueous sodium thiosulfate, dried, and evaporated to give 0.664 g (94%) of **43** as fawn needles: mp, sublimes; UV, IR, ^1H NMR, EIMS.[4] Anal. Calcd for $C_{12}H_6{}^{79}BrN_3$: mol wt, 270.9745. Found: mol wt, 270.9748 (HREIMS).

6-Bromo-1-[3-(1,3-dioxa-2-cyclohexyl)propanoyl]-β-carboline (46). According to the procedure employed for **44**, a solution of **43** (2.0 g, 7.35 mmol) in THF (50 mL) was treated with the 2-(1,3-dioxa-2-cyclohexyl)ethyl Grignard reagent (15.6 mL). Workup and crystallization from methanol/chloroform gave 2.112 g (74%) of **46** as yellow needles: mp 214–216 °C; UV, IR, ^1H NMR, EIMS.[4] Anal. Calcd for $C_{18}H_{17}{}^{79}BrN_2O_3$: mol wt, 388.0422. Found: mol wt, 388.0424 (HREIMS).

6-Bromo-1-(pyrrol-2-yl)-β-carboline (Neoeudistomin 2, 19). According to the procedure employed for **18**, a solution of **46** (2 g, 5.1 mmol) in acetic acid (40 mL) was heated at reflux with ammonium acetate (4 g). Workup and purification by silica gel column chromatography gave 1.469 g (92%) of **19** as a yellow microcrystalline solid: mp 156–157 °C [from $(C_2H_5)_2O/C_6H_{14}$]; UV, IR, ^1H NMR, EIMS.[4] Anal. Calcd for $C_{15}H_{10}{}^{79}BrN_3$: mol wt, 311.0058. Found: mol wt, 311.0056 (HREIMS).

6-Bromo-1-[1-amino-3-(1,3-dioxa-2-cyclohexyl)propyl]-β-carboline (51). According to the procedure employed for **49**, a solution of **43** (2.72 g, 10 mmol) in THF (75 mL) was treated with the 2-(1,3-dioxa-2-cyclohexyl)ethyl Grignard reagent (22 mL) and reduced with sodium borohydride (1 g) in methanol (75 mL). Workup as before, followed by silica gel column chromatography, eluting with 10% methanol in chloroform, gave 3.07 g (79%) of **51** as a glass: UV, IR, ^1H NMR, EIMS.[4] Anal. Calcd for $C_{18}H_{20}{}^{79}BrN_3O_2$: mol wt, 389.0739. Found: mol wt, 389.0740 (HREIMS).

6-Bromo-1-(1-pyrrolin-5-yl)-β-carboline (54). According to the procedure employed for **52**, a solution of **51** (0.10 g, 0.26 mmol) was treated with 70% perchloric acid (1 mL) in water (3 mL) and THF (1 mL). Workup and purification as before gave 0.04 g (50%) of **54** as a glass: UV, IR, ^1H NMR, EIMS.[4] Anal. Calcd for $C_{15}H_{12}{}^{79}BrN_3$: mol wt, 313.0214. Found: mol wt, 313.0206 (HREIMS).

6-Bromo-1-(pyrrolidin-2-yl)-β-carboline (57). According to the procedure employed for **55**, a solution of **54** (0.058 g, 0.18 mmol) in acetic acid (2 mL) and THF (2 mL) was reduced with borane–trimethylamine

(22) McLean, S.; Murray, D. G. *Can. J. Chem.* **1972**, *50*, 1478–1485.
(23) Veeraraghavan, S.; Popp, F. D. *J. Heterocycl. Chem.* **1981**, *18*, 909–915.

complex (0.03 g, 0.41 mmol). Workup and purification as before gave 0.036 g (62%) of **57** as a colorless microcrystalline solid: mp 166–169 °C; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{15}H_{14}{}^{79}BrN_3$: mol wt, 315.0371. Found: mol wt, 315.0370 (HREIMS).

6-Bromo-1-(1-pyrrolin-2-yl)-β-carboline (Eudistomin H, 8). According to the procedure employed for **9**, a solution of **57** (0.128 g, 0.41 mmol) in methanol (10 mL) was oxidized with 5.25% sodium hypochlorite solution (0.6 mL) and anhydrous sodium carbonate (0.2 g). Workup and purification by silica gel column chromatography as before gave 0.102 g (80%) of **8** as colorless needles: mp 146.5–148 °C; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{15}H_{12}{}^{79}BrN_3$: mol wt, 313.0214. Found: mol wt, 313.0212 (HREIMS).

1-Carbomethoxy-6-methoxy-1,2,3,4-tetrahydro-β-carboline (36) Hydrochloride. According to the procedure employed for **35** hydrochloride, a solution of **34**[5] (4.33 g, 17.6 mmol) was esterified in methanol (150 mL) saturated with dry hydrogen chloride. Crystallization from methanol/diethyl ether gave 5.07 g (97%) of **36** hydrochloride as pale green needles: mp 163–166 °C; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{14}H_{16}N_2O_3$: mol wt, 260.1161. Found: mol wt, 260.1165 (HREIMS).

1-Carbamoyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline (38). According to the procedure employed for **37**, **36** hydrochloride (5.23 g, 17.6 mmol) was treated with concentrated ammonia (150 mL). Filtration of the reaction mixture gave 3.208 g (77%) of **38** as a yellow microcrystalline solid: mp 175–177 °C; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{13}H_{15}N_3O_2$: mol wt, 245.1164. Found: mol wt, 245.1166 (HREIMS).

1-Carbamoyl-6-methoxy-β-carboline (40). According to the procedure employed for **39**, a solution of **38** (3.39 g, 13.7 mmol) in xylene (400 mL) was heated at reflux for 2 h with 5% palladium on charcoal (2 g). Crystallization from xylene gave 2.318 g (70%) of **40** as pale yellow needles: mp, sublimes; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{13}H_{11}N_3O_2$: mol wt, 241.0851. Found: mol wt, 241.0850 (HREIMS).

1-Cyano-6-methoxy-β-carboline (42). Phosphorus oxychloride (1.7 mL, 18.2 mmol) was added dropwise over 5 min to a solution of **40** (3.5 g, 14.5 mmol) in dry dimethylformamide (100 mL) and dry pyridine (5 mL), cooled in an ice-water bath. The reaction mixture was stirred for 1 h and then was poured onto ice and extracted from aqueous ammonia with chloroform. Workup and recrystallization from acetic acid gave 2.094 g (65%) of **42** as a yellow microcrystalline solid: mp, sublimes; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{13}H_9N_3O$: mol wt, 223.0746. Found: mol wt, 223.0747 (HREIMS).

1-[3-(1,3-Dioxa-2-cyclohexyl)propanoyl]-6-methoxy-β-carboline (45). According to the procedure employed for **44**, a solution of **42** (0.5 g, 2.24 mmol) in THF (20 mL) was treated with the 2-(1,3-dioxa-2-cyclohexyl)ethyl Grignard reagent (5 mL). After workup, the crude product was purified by column chromatography (silica gel, $CHCl_3$) to give 0.574 g (75%) of **45** as bright yellow needles: mp 144–146 °C; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{19}H_{20}N_2O_4$: mol wt, 340.1422. Found: mol wt, 340.1421 (HREIMS).

6-Methoxy-1-(pyrrol-2-yl)-β-carboline (47). According to the procedure employed for **18**, a solution of **45** (0.5 g, 1.47 mmol) in acetic acid (10 mL) was treated with ammonium acetate (1 g). Workup and purification by silica gel chromatography as before gave 0.26 g (67%) of **47** as yellow-brown prisms: mp 154–156 °C; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{16}H_{13}N_3O$: mol wt, 263.1059. Found: mol wt, 263.1057 (HREIMS).

6-Hydroxy-1-(pyrrol-2-yl)-β-carboline (Eudistomin M, 13). Boron tribromide in dichloromethane (1 M, 1 mL) was added to a solution of **47** (0.05 g, 0.19 mmol) in dichloroethane (5 mL). The reaction mixture was heated at reflux for 0.5 h and then cooled, and the excess reagent was destroyed with methanol. The product was extracted from aqueous ammonia with chloroform and purified by column chromatography (silica gel, 0–2% CH_3OH in $CHCl_3$) to give 0.034 g (72%) of **13** as yellow prisms: mp 225–227 °C (from $CH_3OH/CHCl_3$); UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{15}H_{11}N_3O$: mol wt, 249.0902. Found: mol wt, 249.0909 (HREIMS).

1-[1-Amino-3-(1,3-dioxa-2-cyclohexyl)propyl]-6-methoxy-β-carboline (50). According to the procedure employed for **49**, a solution of **42** (0.2 g, 0.9 mmol) in dry THF (8 mL) was treated with the 2-(1,3-dioxa-2-cyclohexyl)ethyl Grignard reagent (2 mL), and the intermediate imine was reduced with sodium borohydride (0.1 g) in methanol (8 mL). Workup and purification by chromatography [silica gel; 0–5% CH_3OH in $CHCl_3$, followed by $NH_3/CH_3OH/CHCl_3$ (0.5:9.5:90)] gave 0.209 g (68%) of **50** as pale yellow prisms: mp 135–137 °C; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{19}H_{23}N_3O_3$: mol wt, 341.1739. Found: mol wt, 341.1734 (HREIMS).

6-Methoxy-1-(1-pyrrolin-5-yl)-β-carboline (53). According to the procedure employed for **52**, a solution of **50** (1.5 g, 4.4 mmol) in THF (25 mL) was treated with 17.5% aqueous perchloric acid (75 mL). Workup and purification by silica gel column chromatography as before gave 0.798 g (68%) of **53** as a glass: UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{16}H_{15}N_3O$: mol wt, 265.1215. Found: mol wt, 265.1211 (HREIMS).

6-Methoxy-1-(pyrrolidin-2-yl)-β-carboline (56). According to the procedure employed for **55**, a solution of **53** (0.1 g, 0.38 mmol) in THF (4 mL) and acetic acid (4 mL) was reduced with borane–trimethylamine complex (0.03 mg). Workup and purification by silica gel column chromatography as before gave 0.078 g (77%) of **56** as a white microcrystalline solid: mp 162–165 °C; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{16}H_{17}N_3O$: mol wt, 267.1372. Found: mol wt, 267.1372 (HREIMS).

6-Methoxy-1-(1-pyrrolin-2-yl)-β-carboline (58). According to the procedure employed for **9**, a solution of **56** (0.03 g, 0.11 mmol) in methanol (3 mL) was oxidized with 5.25% sodium hypochlorite solution (0.18 mL) and anhydrous sodium carbonate (0.05 g). Workup and purification by silica gel column chromatography gave 0.022 g (74%) of **58** as yellow plates: mp 164–165 °C (from CH_2Cl_2/C_6H_{14}); UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{16}H_{15}N_3O$: mol wt, 265.1215. Found: mol wt, 265.1211 (HREIMS).

6-Hydroxy-1-(1-pyrrolin-2-yl)-β-carboline (Eudistomin Q, 17). Boron tribromide in dichloromethane (1 mL, 1 M) was added to a solution of **58** (0.05 g, 0.19 mmol) in dichloroethane (5 mL). The reaction mixture was heated at reflux for 10 min, then cooled and worked up, and chromatographed as for **13** to give 0.011 g (23%) of **17** as a yellow microcrystalline solid: mp, sublimes; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{15}H_{13}N_3O$: mol wt, 251.1059. Found: mol wt, 251.1053 (HREIMS).

9-(3-Carbethoxypropyl)-1-cyano-β-carboline (60). To a solution of **41** (0.482 g, 2.5 mmol) in dry dimethylformamide (10 mL) was added 0.132 g (2.75 mmol) of 50% sodium hydride in oil. The reaction mixture was stirred for 1 h, and then 0.536 g (2.75 mmol) of ethyl γ-bromobutyrate was added and the reaction mixture was heated at 120 °C for 1.5 h. Water was added and the mixture was extracted with chloroform. Workup and purification by column chromatography on silica, eluting with chloroform, gave an oil, which was crystallized from diethyl ether/60–80 °C petroleum ether to give 0.633 g (83%) of **60** as pale yellow prisms: mp 74–75 °C; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{18}H_{17}N_3O_2$: mol wt, 307.1309. Found: mol wt, 307.1315 (HREIMS).

4-Amino-5-carbethoxy-6,7-dihydro-3,7a-diazacyclohepta[*jk*]fluorene (61). To a solution of **60** (1 g, 3.26 mmol) in toluene (300 mL) was added 1 g (20.8 mmol) of 50% sodium hydride in oil. The reaction mixture refluxed for 3 h, and then water was added and the mixture was extracted with chloroform. Workup and purification by column chromatography over silica gel, eluting with chloroform, with recrystallization from diethyl ether/40–60 °C petroleum ether gave 0.699 g (70%) of **61** as yellow needles: mp 125–126 °C; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{18}H_{17}N_3O_2$: mol wt, 307.1314. Found: mol wt, 307.1317 (HREIMS).

4,5,6,7-Tetrahydro-3,7a-diazacyclohepta[*jk*]fluoren-4-one (62) Hydrobromide. A solution of **61** (0.50 g, 1.63 mmol) in 48% hydrobromic acid (25 mL) refluxed for 2 h. Addition of 25 mL of water gave 0.461 g (89%) of **62** hydrobromide as yellow needles: mp >280 °C; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{15}H_{12}N_2O$: mol wt, 236.0942. Found: mol wt, 236.0946 (HREIMS).

4,5,6,7-Tetrahydro-3,7a-diazacyclohepta[*jk*]fluoren-4-one Oxime (63). Hydroxylamine hydrochloride (0.050 g, 1.1 equiv) was added to the free base from 0.20 g (0.63 mmol) of **62** hydrobromide dissolved in methanol (15 mL). The solution was stirred at room temperature overnight and then made basic with ammonia and extracted into chloroform. Workup gave 0.146 g (92%) of **63** as yellow prisms: mp 174–175 °C; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{15}H_{13}N_3O$: mol wt, 251.1054. Found: mol wt, 251.1056 (HREIMS).

1-Acetamido-4,5,6,7-tetrahydro-3,7a-diazacyclohepta[*jk*]fluorene (59). To **63** (100 mg, 0.398 mmol) in acetic acid (0.3 mL) and acetic anhydride (0.1 mL) was added zinc dust (100 mg) with stirring for 0.75 h at 50 °C. The zinc was removed by filtration and washed with chloroform. The combined organic layers were evaporated, and the crude product was purified by column chromatography over silica gel, eluting with methanol/chloroform (5:95). The resultant oil crystallized on standing to give 97 mg (87%) of **59** as fawn prisms: mp 147–149 °C; UV, IR, ^{1}H NMR, EIMS.[4] Anal. Calcd for $C_{17}H_{17}N_3O$: mol wt, 279.1375. Found: mol wt, 279.1373 (HREIMS).

Acknowledgment. The Alpha Helix Expedition 1978 was supported by a grant from the National Science Foundation (PCM 77-12584) and post-expedition studies in Urbana by the National Institute of Allergy and Infectious Diseases (AI 04769). Mass spectrometry was provided in part by grants from the National Institute of General Medical Sciences (GM 27029) and the National Science Foundation (PCM-8121494) and NMR spec-

troscopy in part by the University of Illinois NSF Regional Instrumentation Facility (NSF CHE 79-16100). We thank J. C. Cook and M. K. Cochran for mass spectra, Drs. N. S. Scott and D. W. Phillipson for NMR spectra, all at the University of Illinois, and Dr. W. C. Krueger and M. D. Prairie, The Upjohn Company, for CD spectra. We also thank the governments of Mexico and Belize for permission to carry out scientific research in their territorial waters and Dr. M. E. Rice and her associates at the Smithsonian Tropical Research Center, Fort Pierce, Florida, for their help and the use of their facilities.

Supplementary Material Available: General experimental section; IR, MS, NMR, and/or UV data for compounds **1–26**, **29–32**, **35–38**, **40**, **42–47**, **49–65**, 4-bromo-2-nitrotoluene, and 6-bromoindole-3-glyoxylamide; Table I, UV Data for Eudistomins and Their Derivatives; Table II, ^{1}H NMR Data for Eudistomins and Their Derivatives; Table III, ^{13}C NMR Chemical Shifts of **1**, **7–9**, **64**, and **65**; Figure 1, COSY of Diacetyleudistomin E (**65**); Figure 2, CD Spectra of Diacetyleudistomins C and E (**64** and **65**); and an isolation flow chart of *E. olivaceum* (22 pages). Ordering information is given on any current masthead page.

The Chiral Bilayer Effect Stabilizes Micellar Fibers

Jürgen-Hinrich Fuhrhop,*† Peter Schnieder,†‡ Jörg Rosenberg,† and Egbert Boekema‡

Contribution from the Institut für Organische Chemie der Freien Universität, Berlin, 1000 Berlin 33, West Germany, and Fritz-Haber-Institut der Max-Planck Gesellschaft, Faradayweg 4-6, 1000 Berlin 33, West Germany. *Received April 24, 1986*

Abstract: Dihelical fibers several micrometers in length and gels were obtained by spontaneous aggregation of octyl L- and D-gluconamides. The single strands have the thickness of a bimolecular layer. No fibers are formed from the racemate. The tendency of the chiral amphiphiles to aggregate to very long fibers instead of three-dimensional crystals is rationalized with a "chiral bilayer effect". This effect is caused by the slowness of rearrangements from tail-to-tail hydrophobic bilayers to crystals, in which the molecular sheets are arranged in a head-to-tail fashion. Thermograms which indicate slow rearrangements in ageing gels are also reported.

The hydrophobic effect[1] impresses sheet-like bilayer structures on the aggregates of water-insoluble amphiphile molecules of cylindrical shape.[2,3] These bilayers may align to form myelin figures[4] or rearrange to spherical vesicle membranes.[5] If the head groups of amphiphiles (i) are chiral and (ii) contain an amide bond, helical fibers and gels may be formed from vesicular or micellar solutions.[6,7] It appears that the formation of essentially linear hydrogen bonds between the amide groups is responsible for this rearrangement.[7] Arnett and Thomson have demonstrated enantiomer discrimination in two-dimensional monolayers of chiral stereamides.[8] In organic solvents helical aggregates of chiral non-amide amphiphiles have also been observed.[9,10] In these cases the corresponding racemate did not produce fibers but platelets.

We are interested in linear aggregates in aqueous media, because they constitute the structural counterpart to spherical vesicles. A combination of both may produce vesicle membranes with protrusions and/or ordered gel structures in the inner volume. This is considered as an important synthetic step toward functional cell models.[3]

In this paper we describe a new type of "bulgy double helix" made from *N*-alkylgluconamides in water. The single strands of these helices are as thin as molecular bilayers, which can only be arranged in tail-to-tail fashion. Anhydrous crystals of *N*-alkyl-D-gluconamides, however, show head-to-tail (or enantiopolar) packings of adjacent molecular sheets.[11] This structural phenomenon is used to introduce a new "chiral bilayer effect", which explains the longevity of chiral fibers and gels as compared to racemic analogues, which precipitate as crystals.

Experimental Section

Syntheses of Gluconamides. The D-gluconamides **1a** and **1b** were obtained by aminolysis of D-glucono-δ-lactone (Sigma, Deisenhofen) with *n*-octylamine or *n*-docecylamine in methanol. L-Glucono-γ,δ-lactones were prepared by indirect electrolytic oxidation in the presence of calcium bromide and calcium carbonate.[12,13] Excess bromide was precipitated with silver carbonate. The filtered solution was treated with a strongly acidic ion exchange resin (Merck) and dehydrated by azeotropic destillation with 1-butanol. The yield of L-glucono-γ,δ-lactones was 80%, the final aminolysis with octylamine occurred quantitatively. mp (**1a**) = 158°C, (**2**) 156°C, **1a+2** (1:1) 154.5°C; $[a]^{25}D$(1a) = +28.3° (in Me_2SO); $[\alpha]^{25}_D$(**2**) = −27.1° (in Me_2SO); spectra (IR, ^{1}H NMR, and MS) and elemental analyses (C, H, N) are added as supplementary material.

Gels and Electron Micrographs. Gels were formed by heating **1a** or **1b** or **2** in water to 100 °C and cooling to room temperature. They were obtained in the concentration range from 0.5 to 50% (w/v). Below 0.5% incoherent gel flakes in fluid water were formed. Above 50% the solution remained turbid at 100 °C. The gels remained clear for a few hours. After a day crystals began to separate.

At pH 2 and in the presence of 2% phosphotungstic acid, however, 2–20% (w/v) gels remained clear for weeks. This behavior has also been observed for polysaccharide gels.[14] Electron microscopy was carried out with a Philips EM 300 at 80 kV and direct magnification of 70 000.

Negatively stained samples were prepared by dipping carbon-coated

* Address correspondence to this author.
† Institut für Organische Chemie der Freien Universität.
‡ Fritz-Haber-Institut der Max-Planck Gesellschaft.

(1) Fendler, J. H., *Membrane Mimetic Chemistry*; Wiley: New York, 1682.
(2) Eibl, H. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 257.
(3) Fuhrhop, J.-H.; Mathieu, J., *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 100–113.
(4) Stoeckenius, W., *J. Biophys. Biochem. Cytol.* **1959**, *5*, 491–505.
(5) Reference 1, p 115.
(6) Nakashima, N.; Asakuma, S.; Kunitake, T. *J. Am. Chem. Soc.* **1985**, *107*, 509–510.
(7) Pfannemüller, B.; Welte, W. *Chem. Phys. Lipids* **1985**, *37*, 227–240.
(8) Arnett, E. M.; Thomson, O. *J. Am.* Chem. *Soc.* **1981**, *103*, 968–970.
(9) Tachibana, T.; Yoshizumi, T.; Hori, K. *Bull, Chem. Soc. Jpn.* **1979**, *52*, 34–41.
(10) Hidaka, H.; Murata, M.; Onai, T. *J. Chem. Soc., Chem. Commun.* **1986**, 562–564.
(11) Zabel, V.; Müller-Fahrnow, A.; Hilgenfeld, R.; Saenger, W.; Pfannemüller, B.; Enkelmann, V.; Welte, W. *Chem. Phys. Lipids* **1986**, *39*, 313–327 and references therein.
(12) Frush, H. L.; Isbell, H. S. *Methods Carbohydr. Chem.* **1963**, *2*, 14–18.
(13) Emmerling, W. N.; Pfannemüller, B. *Makromol. Chem.* **1978**, *179*, 1627–1633.
(14) Hayat, M. A. *Principles and Techniques of Electron Microscopy*; van Nostrand Reinhold: New York, 1970; p 272, pp 274ff.

0002-7863/87/1509-3387$01.50/0