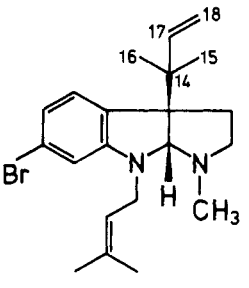
Bromo-Substituted Physostigmine Alkaloids from a Marine Bryozoa *Flustra foliacea*

Sir:

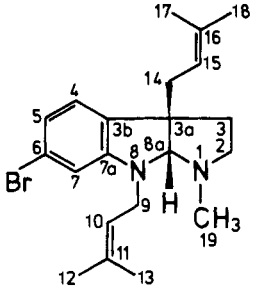
Among the ever-increasing number of organic structures emerging from marine natural products research, extremely few belong to the alkaloids.[1] This has given rise to the assumption that marine organisms have evolved no biosynthetic pathways comparable to the ones which in terrestrial organisms lead to the amazing diversity of structures constituting the alkaloids. A very limited number of the known marine alkaloids are bromo compounds; however, these seem to bear no resemblance to structures reported from terrestrial sources. We now wish to report on the isolation and structure elucidation of two brominated alkaloids, which, although possessing unusual substitution patterns, clearly are referable to the group of alkaloids derived from the physostigmine skeleton.

During a search for chemical transmitters in the marine environment,[2] we examined the marine Bryozoa *Flustra foliacea* (L.). Although one of the major animal phyla (approximately 4000 living species), Bryozoa (syn. Ectoprocta, Polyzoa, or moss animals) seem to have escaped the attention of the natural products chemists, although from an economic point of view Bryozoa are one of the most important groups of fouling organisms.[3]

In continuation of our study of volatile compounds from *F. foliacea* (L.),[2] the nonpolar constituents of the animals were investigated. From a petroleum ether extract of freeze-dried material two isomeric alkaloids, designated flustramine A (**1**) and flustramine B (**2**), were secured. It is noteworthy that both alkaloids encompass the unprecedented bromophysostigmine skeleton and a rare N-8 substituent, namely, γ,γ-dimethylallyl. Furthermore, flustramine A carries the unusual feature



1. FLUSTRAMINE A



2. FLUSTRAMINE B

of an inverted isoprene unit (2-methyl-3-buten-2-yl) at the 3 position of the indoline nucleus, in contrast to flustramine B, which carries a γ,γ-dimethylallyl unit at this position.

The freeze-dried animals yielded 1.6% of petroleum ether soluble material. A mixture of the alkaloids (0.07% based on dry weight) was isolated following column chromatography (silica gel, MeOH:CH_2Cl_2, 5:95). The separation of **1** (50%) and **2** (50%) was achieved using silica gel chromatography, with ethyl acetate as eluent.

The elemental compositions $C_{21}H_{29}BrN_2$ were determined from high resolution MS (calcd 388.151; found for **1**, 388.150, and **2**, 388.151). An important clue to the structures was gathered from the abundant fragment ions at *m/e* 208 (16 and 12% for **1** and **2**, respectively) still containing the characteristic bromine isotopic pattern. This ion (corrected for bromine content) is indicative of an indole subunit, which in this case is believed to represent the bromo-3-methylindoline fragment ion.[4]

High resolution data reveal the ions at *m/e* 319 (**1**, 52%; **2**, 28%) and 251 (**1**, 80%; **2**, 20%) to correspond to loss of C_5H_9 and C_5H_8 fragments, viz. the two isoprene units. The cyclic collapse of the physostigmine skeleton giving rise to *m/e* 208 not only places the bromine atom in the indoline part of the molecule, but also locates the *N*-methyl group at N-1.[4]

A crucial point in the structural analysis, namely, the demonstration of the indoline subunit, was overcome by MCD techniques. As shown by Djerassi et al.,[5] MCD will distinguish between the indole and the indoline chromophore in the 250–330-nm wavelength region, since compounds containing an indole subunit invariably show a −+ sign sequence, whereas those derived from indoline show a +− sign sequence in the MCD curves. In the case of flustramine A (**1**), the MCD at 10 kG exhibits bands at 263 nm ($\Delta\epsilon = 8.6 \times 10^{-2}$) and 317 nm ($\Delta\epsilon = -1.2 \times 10^{-1}$), establishing the indoline configuration. Further support for this assignment was derived from the UV spectra (**1**: λ_{max} (EtOH) 208 nm (ϵ 2.0×10^4), 253 (8.6×10^3), and 319 (3.4×10^3)) which upon acidification underwent the expected hypsochromic shift of 10 nm.[6]

Having thus secured the basic physostigmine skeleton, the positions of the isoprene units were determined. As neither IR nor ^{1}H NMR spectroscopy shows any indication of a N–H grouping (e.g., the ^{1}H NMR spectrum exhibits no protons exchangable with deuterium, CD_3OD), both compounds must carry isoprene substituents at the indoline nitrogen atoms, since N-1 is methylated (vide supra). Comparison of the pertinent 270 MHz ^{1}H NMR data for **1** [($CDCl_3$) δ 5.18 (t, 1 H at C-10, J = 6.3 Hz), 3.84 (d, 2 H at C-9, J = 6.3 Hz), 1.73 (s, 3 H at C-12 or C-13), 1.72 ppm (s, 3 H at C-12 or C-13)] and **2** with those reported for fumitremorgin B[7] (=lanosulin[8]) [($CDCl_3$) δ 5.04 and 4.52 ppm] substantiated the assignment of a γ,γ-dimethylallyl grouping at N-8 in **1** and **2**.

The position of the last isoprene unit was inferred from the sharp singlet proton resonances at δ 4.37 and 4.31 ppm in **1** and **2**, respectively; since these signals originate from the 8a hydrogen atom, it follows that C-3a must be quaternary, thus bearing the isoprene substituent. In the case of **1** this substituent was assigned the inverted γ,γ-dimethylallyl structure based on the C-15 and C-16 methyl signals at δ 0.95 and 1.01 ppm, and on the ABZ pattern of the three vinylic protons appearing between δ 5.18 and 5.90 ppm. These data are all in excellent agreement with data published for this structural subunit (see, e.g., roquefortine[9] and oxaline[10]). In compound **2**, data for the 3a substituent includes an AB pattern between δ 4.92 and 2.39 ppm (J_{AB} = 7.2 Hz) and two methyl group singlets appearing at δ 1.65 and 1.57 ppm, again in close agreement with literature data for related systems[11] bearing γ,γ-dimethylallyl at position 3a. The assignment of proton resonances was, whenever possible, verified by extensive decoupling experiments, which also served to entangle the signals belonging to a given structural unit.[12] The substitution of an inverted γ,γ-dimethylallyl group in **1** with a γ,γ-dimethylallyl group in **2** is consistent with the replacement of ^{13}C NMR signals at δ 144.9 (C-17) and 113.1 ppm (C-18) observed for **1**, appearing at the expected values of δ 120.9 and 134.6 ppm, respectively, in the spectrum of **2**.

Inspection of the aromatic region of the ^{1}H NMR spectrum (**1**: δ 6.87, 6.67, 6.47 ppm; $J_{4,5}$ = 8.3, $J_{4,7}$ = 1.5 Hz) did not reveal the position of the bromine atom. Using the ^{13}C NMR chemical shifts for the physostigmine skeleton,[13] it was possible to locate the bromine at the 6 position. Predicted chemical shifts for a hypothetical C-5 brominated isomer were not compatible with the experimental data at all. Additional evidence for this assignment was obtained from NOE difference spectroscopy, which allows NOE detected in the Fourier transform mode to be observed.[14] Irradiation with the frequency corresponding to the resonance of the C-9 protons produced an enhancement of 11% in the signal appearing at δ 6.47 ppm defining this as the resonance of the proton at C-7 and thus (vide supra) unequivocally placing the bromine at position 6.[15] This is another example of the rule of thumb that monobrominated derivatives of tryptophan of marine origin

0002-7863/79/1501-4012$01.00/0

carries the bromo substituent at the 6 position[16] reflecting presumably some biosynthetic pathway still to be elucidated.

Acknowledgment. The Bruker HX-270 NMR spectrometer was purchased by Danish Natural Science Research Council to whom we are also grateful for a fellowship (No. 511-100166) to one of us (J.S.C.). We wish to thank Dr. J. Øgaard Madsen for the mass spectral data, Dr. E. Larsen for the MCD spectra and Dr. Ole Manscher for helpful discussions.

References and Notes

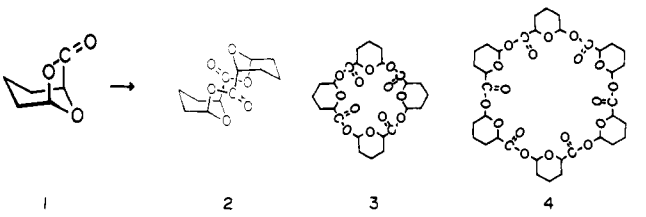
(1) R. H. Thomson, *Chem. Br.*, **14**, 133 (1978).
(2) C. Christophersen and J. S. Carle, *Naturwissenschaften*, **65**, 440 (1978).
(3) R. D. Barnes, "Invertebrate Zoology", 2nd ed., W. B. Saunders, Philadelphia, 1968, pp 588–599.
(4) H. Budziekiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry", Vol. 1, Holden-Day Inc., San Francisco, 1964.
(5) G. Barth, R. E. Linder, E. Bunnenberg, and C. Djerassi, *Helv. Chim. Acta*, **55**, 2168–2178 (1972).
(6) H. F. Hodson and G. F. Smith, *J. Chem. Soc.* 1877 (1957).
(7) M. Yamazaki, K. Sasago, and K. Miyaki, *J. Chem. Soc., Chem. Commun.*, 408 (1974).
(8) D. T. Dix, J. Martin, and C. E. Moppett, *J. Chem. Soc., Chem. Commun.*, 1168 (1972).
(9) P. M. Scott, M.-A. Merrien, and J. Polonsky, *Experientia*, **32**, 140 (1976).
(10) D. W. Nagel, K. G. R. Pachler, P. S. Steyn, P. L. Wessels, G. Gafner, and G. J. Kruger, *J. Chem. Soc., Chem. Commun.*, 1021 (1974).
(11) G. Casnati, M. Francioni, A. Guareschi, and A. Pochini, *Tetrahedron Lett.*, 2485 (1969).
(12) Complete experimental details and data together with full ^1H and ^{13}C NMR assignments will appear in a forthcoming full paper.
(13) P. A. Crooks, B. Robinson, and O. Meth-Cohn, *Phytochemistry*, **15**, 1092 (1976).
(14) See, e.g. R. Richarz and K. Wüthrich, *J. Magn. Reson.*, **30**, 147 (1978); G. E. Chapman, B. D. Abercrombie, P. D. Cary, and E. M. Bradbury, *ibid.*, **31**, 459 (1978).
(15) This technique also demonstrated the relative stereochemistry of the five-membered rings in **2** to be cis (like physostigmine) since a 3% enhancement was observed for the proton 3a on irradiation of the protons situated at C-14.
(16) See, e.g., P. J. Scheuer, "Chemistry of Marine Natural Products", Academic Press, New York, 1973.

Jørgen S. Carlé, Carsten Christophersen
Marine Chemistry Section, University of Copenhagen
The H. C. Ørsted Institute, DK-2100 Copenhagen, Denmark
Received March 27, 1979

A Novel 30-Membered Synthetic Macrolide from (+)-(1*R*,5*R*)-6,8-Dioxabicyclo[3.2.1]octan-7-one

Sir:

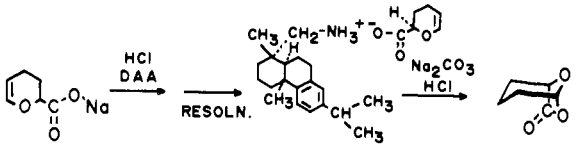
Recent advances in the chemistry of crown ethers and cryptands have stimulated many groups of investigators to design macrocyclic ligands for molecular recognition and synthetic ionophores with biological functionality. In the course of the studies on the ring-opening polymerization of bicyclic compounds containing a tetrahydropyran ring, we found that racemic 6,8-dioxabicyclo[3.2.1]octan-7-one (**1**) underwent cationic polymerization at low temperature to provide 10-, 20-, and 30-membered cyclic oligoesters (**2–4**) consisting of alternating tetrahydropyran and ester moieties,[1,2] which bear structural resemblance to naturally occurring antibiotics, nonactin and its analogues. Furthermore, each of these cyclic oligomers was formed selectively, even nearly quantitatively for the cyclic dimer and tetramer, by proper choice of reaction conditions.[2,3]



1 2 3 4

These intriguing findings prompted us to investigate the oligomerization of an optically active monomer, in expectation of obtaining macrocyclic oligoesters with well-defined configuration. The present communication describes convenient synthetic procedures for (+)-(1*R*,5*R*)-6,8-dioxabicyclo[3.2.1]octan-7-one and its cyclic hexamer, a 30-membered synthetic macrolide.

(+)-(1*R*,5*R*)-6,8-Dioxabicyclo[3.2.1]octan-7-one (**7**) was successfully prepared through the optical resolution of racemic 3,4-dihydro-2*H*-pyran-2-carboxylic acid by using dehydroabietylamine, a base which has lately been used with success in optical resolution of several carboxylic acids.[4,5] An aqueous solution of sodium 3,4-dihydro-2*H*-pyran-2-carboxylate (**5**) was slightly acidified with 6 N hydrochloric acid, and the liberated carboxylic acid was extracted several times with ethyl ether. The ethyl ether extract was then added to an ice-cooled



5 6 7

ethyl ether solution of dehydroabietylamine with occasional shaking. Immediately, a white mass was formed, which was separated and recrystallized repeatedly from methanol solution to yield pure (+)-dehydroabietylammonium 3,4-dihydro-2*H*-pyran-2-carboxylate (**6**) as white needles: $[\alpha]^{24}_D$ +11.7° (*c* 1 g/dL, ethanol); mp 168–171 °C. Anal. ($C_{26}H_{39}NO_3$) C, H, N. The diastereomeric ammonium salt was converted to the sodium salt on treatment with aqueous sodium carbonate, and the liberated dehydroabietylamine was removed by extraction with ethyl ether. The sodium salt was subsequently transformed to the free acid followed by immediate distillation under reduced pressure to afford an optically active monomer **7** with $[\alpha]^{24}_D$ +128° (*c* 1.0 g/dL, ethanol); bp 61 °C (5 mm).

The ammonium carboxylate **6** was converted to the sodium carboxylate, which was then esterified with ethyl iodide in dimethylformamide. Subsequent reduction of the ethyl ester with lithium aluminum hydride in ethyl ether gave 2-hy-

Table I. Oligomerization of (+)-(1*R*,5*R*)-6,8-Dioxabicyclo[3.2.1]octan-7-one[a]

monomer, g	solvent,[b] mL	initiator, mol %	time, h	conversion, %[c] dimer	tetramer	hexamer	other	total
0.5	MC, 0.5	1	48	0	5	63	7	75
1.0	AN, 1.0	1	24	0	25	59	0	84
1.0	CF, 1.0	5	24	0	0	46	0	46
1.0	NP, 1.0	1	24	0	0	81	0	81

[a] Initiator, BF_3OEt_2; temperature, −40 °C. [b] MC, methylene chloride; AN, acetonitrile; CF, chloroform; NP, 1-nitropropane. [c] Determined by gel permeation chromatography.

0002-7863/79/1501-4013$01.00/0