Synthesis of Cyclic Glycerol Acetal Phosphates: Proton and Carbon-13 NMR Characteristics of Isomeric 1,3-Dioxolane and 1,3-Dioxane Phosphate Structures

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Structural and geometrical isomers of long-chain cyclic glycerol acetal phosphates were synthesized from the respective 2-pentadecyl-*cis*- and -*trans*-4-(hydroxymethyl)-1,3-dioxolanes **1** and 2-pentadecyl-*cis*- and -*trans*-5-hydroxy-1,3-dioxanes **5** by phosphorylation with diphenyl phosphorochloridate followed by hydrogenolysis. The reactions proceeded in an essentially quantitative fashion. The stereochemistry of the cis/trans isomeric five- and six-membered-ring cyclic glycerol acetal phosphates **3** and **7** was established by ^1H and ^{13}C nuclear magnetic resonance, and retention of configurations throughout the reaction steps was verified for the stereomeric diphenyl phosphates **2** and **6**, phosphates **3** and **7**, and dimethyl phosphates **4** and **8**. Three-bond $^{31}POC^{13}C$ couplings were found useful for describing prevalent rotamer populations of the isomeric cyclic glycerol acetal phosphates as a function of ring acetal configuration and phosphate substitution.

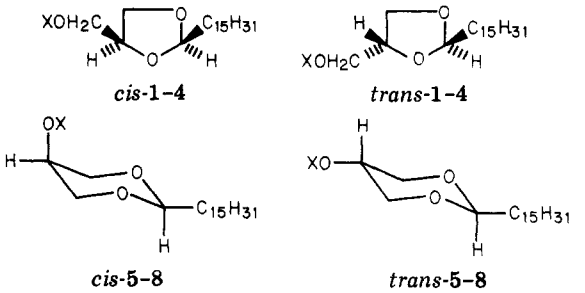
Phosphates of lipophilic cyclic glycerol acetals, as they have been isolated from small intestine ("Darmstoff")[1,2] or kidney medulla extracts ("Kex"),[2,3] evoke or intensify rhythmic smooth muscle contractions reminiscent of those caused by prostaglandins. Yet, unlike prostaglandins, cyclic glycerol acetal phosphates (cyclic GAP) fail to potentiate the effect of acetylcholine on smooth muscle[2b] and they do not possess hypotensive activity,[1g] but cyclic GAP does inhibit pressor responses to renin.[4] The biological activity of cyclic GAP is thought to be mediated by affecting the sodium pump[1e,h] and calcium availability.[1h]

We have previously synthesized long-chain stereomeric cyclic glycerol acetals[5,6] and have characterized these cis and trans isomeric 1,3-dioxolanes[5,6] and 1,3-dioxanes.[5] We now report on the synthesis of specific cyclic glycerol acetal phosphates from individual long-chain glycerol acetals by reaction with diphenyl phosphorochloridate followed by hydrogenolysis and on the configurations and conformations of isomerically pure cyclic GAP as determined by ^1H and ^{13}C NMR. Availability of structurally defined cyclic GAP species should aid in establishing structure–function correlations for these biologically important agents and should enhance present understanding of the stereochemistry of long-chain heterocyclic phosphates in general.

Results and Discussion

The stereomeric 2-pentadecyl-4-(hydroxymethyl)-1,3-dioxolanes eV) *trans*-1), prepared from the respective glycerate acetals,[6] and the stereomeric 2-pentadecyl-5-hydroxy-1,3-dioxanes (*cis*-**5**, *trans*-**5**), prepared by condensation of glycerol with hexadecanal,[5] were reacted with diphenyl phosphorochloridate under conditions similar to those described for the synthesis of enantiomeric phosphatidic acids.[7] Phosphorylation of each of the isomers produced the respective five-membered-ring (*cis*-**2**, *trans*-**2**) and six-membered-ring (*cis*-**6**, *trans*-**6**) glycerol acetal diphenylphosphoric acid esters (70–90% recovered yield) which were then converted to the glycerol acetal phosphates (*cis*-**3**, *trans*-**3** and *cis*-**7**, *trans*-**7**) by hydrogenation over a platinum catalyst (75–95% yield). Both reaction steps proceeded in an essentially quantitative fashion, but losses occurred during thin-layer chromatographic (TLC)[8] purification. Because of the relative instability of cyclic glycerol acetal phosphates,[10] for further characterization of cyclic GAP, and for assurance of isomeric purities, dimethyl derivatives *cis*-**4**, *trans*-**4** and *cis*-**8**, *trans*-**8** were prepared from the respective cyclic GAP isomers **3** and **7** by methylation[11] with diazomethane (Scheme I).

Scheme I



Mass spectrometry (70 eV) of the glycerol acetal derivatives 1–8 (see Experimental Section) produced the expected molecular ions and gave fragmentation patterns consistent with the cyclic acetal structure.[12] Ions [M –

(1) (a) W. Vogt, *Arch. Exp. Pathol. Pharmakol.*, **206**, 1 (1949); (b) W. Vogt, *ibid.*, **227**, 224 (1955); (c) W. Vogt, *J. Physiol. (London)*, **133**, 64P (1956); (d) W. Vogt, *ibid.*, **137**, 154 (1957); (e) W. Vogt, *Nature (London)*, **179**, 300 (1957); (f) W. Vogt, *Pharmacol. Rev.*, **9**, 407 (1958); (g) W. Vogt, *Arzneim.-Forsch.*, **8**, 253 (1958); (h) W. Vogt, *Biochem. Pharmacol.*, **12**, 415 (1963); (i) G. W. Gray, *J. Pharmacol. Exp. Ther.*, **146**, 215 (1964).

(2) (a) D. D. Sumner, Ph.D. Thesis, University of Kansas, 1969; (b) R. A. Wiley, D. D. Sumner, and E. J. Walaszek, *Lipids*, **5**, 803 (1970).

(3) (a) J. B. Lee, K. Crowshaw, B. H. Takman, K. A. Attrep, and J. Z. Gougoutas, *Biochem. J.*, **105**, 1251 (1967); (b) D. C. Dyer and E. J. Walaszek, *J. Pharmacol. Exp. Ther.*, **160**, 360 (1968).

(4) R. D. Buñag and E. J. Walaszek, *Eur. J. Pharmacol.*, **23**, 191 (1973).

(5) W. J. Baumann, *J. Org. Chem.*, **36**, 2743 (1971).

(6) Y. Wedmid and W. J. Baumann, *J. Org. Chem.*, **42**, 3624 (1977).

(7) E. Baer, *J. Biol. Chem.*, **189**, 235 (1951).

(8) Analytical and preparative thin-layer chromatography (TLC) was done on layers of silica gel H (Merck), 0.3 mm thick, in tanks lined with filter paper. For analytical purposes, fractions were made visible by spraying with a solution prepared from 15 g of potassium dichromate, 30 mL of concentrated H_2SO_4, and 150 mL of water, followed by charring; phosphorus-containing fractions were made visible with molybdenum spray reagent.[9] In preparative work, lipid fractions were visible as opalescent bands; these were scraped off, and the lipids were eluted.

(9) J. C. Dittmer and R. L. Lester, *J. Lipid Res.*, **5**, 126 (1969).

(10) Acetal phosphates **3** and **7**, consisting of an acid-labile and an acidic moiety, can be prepared in anhydrous form by lyophilization from benzene and can be stored as powders at freezer temperature without degradation or isomerization.

(11) O. Renkonen, *Biochim. Biophys. Acta*, **152**, 114 (1968).

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Table I. H-2 NMR Signals of Isomeric 2-Pentadecyl-1,3-dioxolanes 1-4 and 2-Pentadecyl-1,3-dioxanes 5-8[a]

	1,3-dioxolanes				1,3-dioxanes			
isomer	1	2	3	4	5	6	7	8
cis	4.90 (4.5)[b]	4.86 (4.4)	4.89 (4.6)[c]	4.89 (4.5)[d]	4.54 (4.7)	4.55 (4.6)[e]	4.59 (4.7)[c,f]	4.54 (4.7)[d]
trans	5.00 (4.5)[b]	4.91 (4.7)	5.00 (4.2)[c]	4.99 (4.5)[d]	4.39 (4.7)	4.39 (4.6)[e]	4.44 (4.6)[c,f]	~4.4 (4.2)[d]

[a] Proton chemical shifts (δ) downfield from Me_4Si and coupling constants (J, Hz) of the H-2 acetal triplets measured at 79.54 MHz in $CDCl_3$ at 35 ± 1 °C, unless noted otherwise. [b] See also ref 6. [c] Measured in 5:4:1 $CDCl_3$-CD_3OD-D_2O (v/v/v). [d] Dimethyl phosphates **4** and **8** give rise to characteristic CH_3 doublets (6 H) centered at ~3.78 ppm, J_{POCH} = 11.2 Hz as expected for such couplings; see ref 13. [e] Poorly resolved from glycerol protons. [f] Measured at 55 °C to shift overlapping HOD signal upfield.

Table II. ^{13}C NMR Chemical Shifts and ^{13}C-^{31}P Couplings of Isomeric 4-Substituted 2-Pentadecyl-1,3-dioxolanes 1-4[a]

carbon	*cis*-1[b]	*trans*-1[b]	*cis*-2	*trans*-2	*cis*-3[c]	*trans*-3[c]	*cis*-4	*trans*-4
2	105.3	105.1	105.7	105.2	105.7	105.3	105.7	105.2
4	76.4	76.3	73.7 (8.4)	73.7 (7.7)	74.6[d]	74.5[d]	74.1 (6.7)	74.0 (7.6)
5	66.5	66.8	66.9	66.7	67.2	67.1	67.0	66.8
CH_2[e]	63.5	62.9	68.7 (6.1)	68.3 (6.3)	66.9[d]	66.4[d]	67.0 (5.7)	67.2 (6.0)
COP[f]			150.6 (7.2)	150.6 (7.1)			54.4 (5.7)	54.4 (5.7)
C-2,6$_{arom}$[g]			120.1 (4.8)	120.1 (4.8)				
1'[h]	34.0	34.4	33.9	33.9	34.1	34.2	34.0	34.0
2'[h]	24.0	24.0	23.9	23.9	24.3	24.1	24.0	23.9

[a] Chemical shifts (δ) downfield from Me_4Si and coupling constants ($J_{C\text{-}P}$, Hz) in proton-decoupled spectra measured at 20 MHz in $CDCl_3$ at 38 ± 1 °C, unless noted otherwise. Carbon signals characterized by J values appear as doublets due to ^{13}C-^{31}P coupling. In $CDCl_3$, additional aliphatic signals occur at 14.1 ppm (ω CH_3), 22.7 (ω-1 CH_2), 32.0 (ω-2 CH_2), and 29.4-29.7 (methylene envelope).[16] [b] See also ref 6. [c] Measured in 5:4:1 $CDCl_3$-CD_3OD-D_2O (v/v/v); in this solvent, the aliphatic signals are observed at 14.2 (ω CH_2), 22.9-23.0 (ω-1 CH_2), 32.2-32.3 (ω-2 CH_2), and 29.9-30.0 (methylene envelope). [d] $J_{C\text{-}P}$ not measurable due to line broadening. [e] Hydroxymethyl CH_2 in **1**, (diphenylphospho)methyl CH_2 in **2**, phosphomethyl CH_2 in **3**, and (dimethylphospho)methyl CH_2 in **4**. [f] C-1$_{arom}$ in **2**, CH_3 in **4**. [g] Additional aromatic carbon signals occur at 129.8 (C-3,5$_{arom}$) and 125.5 (C-4$_{arom}$).[17] [h] C-1' and C-2' refer to the first and second methylene carbons of the aliphatic chain.

Table III. ^{13}C NMR Chemical Shifts and ^{13}C-^{31}P Couplings of Isomeric 5-Substituted 2-Pentadecyl-1,3-dioxanes 5-8[a]

carbon	*cis*-5	*trans*-5	*cis*-6	*trans*-6	*cis*-7[b]	*trans*-7[b]	*cis*-8	*trans*-8
2	102.9	102.2	102.2	102.3	102.9	102.7	102.4	102.4
4,6	71.8	71.6	69.2 (4.8)	69.1 (6.0)	70.1[c]	69.9[c]	69.5 (4.6)	69.4 (5.9)
5	64.2	61.4	71.6 (5.7)	67.3 (6.0)	69.3[c]	65.3[c]	70.1 (5.1)	66.0 (5.6)
COP[d]			150.5 (7.2)	150.3 (7.1)			54.4 (6.3)	54.5 (5.9)
C-2,6$_{arom}$[e]			120.3 (4.8)	119.9 (4.8)				
1'[f]	35.0	34.3	34.8	34.1	35.0	34.5	34.9	34.2
2'[f]	23.9	24.2	23.7	23.9	24.3	24.4	23.8	24.0

[a] See footnote *a*, Table II. [b] See footnote *c*, Table II. [c] See footnote *d*, Table II. [d] C-1$_{arom}$ in **6**, CH_3 in **8**. [e] See footnote *g*, Table II. [f] See footnote *h*, Table II.

$C_{15}H_{31}]^+$ gave rise to the base peak in the spectra of the diphenyl phosphates **2** and **6** and the dimethyl phosphates *cis*-**4**, *trans*-**4**, and *cis*-**8**. In contrast, *trans*-**8** produced a base peak for $[PO(OCH_3)_2OH_2]^+$, suggesting reduced interaction between the polar head group and the ring oxygen in the *trans*-**8** isomer. Cyclic GAP **3** and **7** produced $[M - C_{15}H_{31} - PO_3H]^+$ as the most abundant fragmentation ions.

Particular care was taken to verify isomeric homogeneity, to substantiate configurational assignments of cyclic acetals **1**-**8** by ^1H and ^{13}C nuclear magnetic resonance spectrometry, and to affirm retention of configuration throughout the synthesis.

^1H NMR spectra of the long-chain 2-alkyl-1,3-dioxolanes **1**-**4** show a number of poorly resolved signals in the 3.5-4.5-ppm range due to the protons of C-4,5 and the 4-substituent methylene group. However, prominent triplets ($J \approx 4.5$ Hz) are observed near 4.9 ppm in the spectra of the cis isomers **1**-**4** and near 5.0 ppm for the trans isomeric structures (Table I). These triplets are assigned to the acetal protons H-2[14] and permit discrimination of cis/trans isomers. Configurational assignments are possible on the basis of the downfield shift observed for H-2 in the case of the trans-substituted 1,3-dioxolanes due to the closer proximity of the ring substituent at C-4 and the acetal proton in the trans isomers **1**-**4** leading to deshielding. Such H-2 shifts to lower field strength have previously been described for other trans 2,4-substituted 1,3-dioxolane systems.[5,6,15]

The ^1H NMR spectra of the long-chain 2-alkyl-1,3-dioxanes **5**-**8** show the H-2 triplet ($J \approx 4.6$ Hz) close to 4.55 ppm in the case of the cis isomers and near 4.4 ppm for the trans isomeric dioxanes[14] (Table I). Both isomers bear H-2 in axial conformation, as is also indicated by the magnitude (~5 Hz) of the couplings observed.[5] Shift differences for H-2 within isomeric pairs are thought to be due to interactions between the axial substituent at C-5 and the ring oxygens in the cis isomers. The proton NMR

(12) W. J. Baumann, A. J. Aasen, J. K. G. Kramer, and R. T. Holman, *J. Org. Chem.*, 38, 3767 (1973).
(13) A. A. Gallo, A. J. Hancock, and H. Z. Sable, *J. Lipid Res.*, 18, 77 (1977).
(14) C-2 assignments were confirmed by specific deuteration at C-2 in the case of *cis*-**1**, *trans*-**1**, *cis*-**5**, and *trans*-**5**.[5,6]
(15) N. Baggett, J. M. Duxbury, A. B. Foster, and J. M. Webber, *J. Chem. Soc. C*, 208 (1966).
(16) (a) J. G. Batchelor, R. J. Cushley, and J. H. Prestegard, *J. Org. Chem.*, 39, 1698 (1974); (b) A. P. Tulloch and M. Mazurek, *Lipids*, 11, 228 (1976).
(17) L. F. Johnson and W. C. Jankowski, "Carbon-13 NMR Spectra", Wiley, New York, 1972.

data demonstrate that configurations were maintained throughout the synthesis of five- and six-membered cyclic GAP (Table I).

Proton-decoupled ^{13}C NMR spectra of the long-chain 2-alkyl-1,3-dioxolanes 1–4 (Table II) and 2-alkyl-1,3-dioxanes 5–8 (Table III) show distinct and discriminatory chemical shift differences for cis/trans isomeric pairs. Carbon 2 assignments were substantiated by specific C-2 deuteration.[14] All assignments of ring carbons 2, 4(6), and 5 in 1–8 and the 4-methylene substituent in 1–4 are consistent with the multiplicities observed in off-resonance proton-decoupling experiments.

A comparison of the **^{13}C chemical shifts** of the ring carbons of the 4-substituted 2-pentadecyl-1,3-dioxolanes 1–4 (Table II) with those of the nonsubstituted 2-pentadecyl-1,3-dioxolanes (C-2 104.9 ppm; C-4,5 64.8 ppm)[6] shows that substitution in position 4 causes deshielding and downfield shifts of 8.9–11.6 ppm for C-4, 1.7–2.4 ppm for C-5, and 0.2–0.8 ppm for the acetal carbon in position 2. In all instances, cis substitution at C-4 produced 0.2–0.5-ppm greater deshielding of C-2 than did trans substitution. C-4 and C-5 chemical shifts were less affected by the configuration of the dioxolane system. Greater shift differences (0.2–0.6 ppm) became apparent for the 4-methylene substituent of cis/trans isomeric dioxolane pairs (Table II). Phosphorylation of the 4-(hydroxymethyl) function in *cis*-**1** (63.5 ppm) and *trans*-**1** (62.9 ppm) in turn produced downfield shifts of 3.4–3.5 ppm in the case of the 4-methylene phosphates **3** and greater deshielding (3.5–5.4 ppm) in the diphenyl- and dimethylphosphomethyldioxolanes (**2** and **4**) depending upon the nature of the substituents and on their orientation relative to the dioxolane ring. Conversely, the phosphate group in **4** (or **8**) exerts a downfield shift of 4.3 ppm on the methyls (54.4 ppm) with respect to the carbon chemical shift of methanol (50.1 ppm, dilute solution in chloroform). These deshieldings by a phosphate on a hydroxylated carbon are more pronounced than those reported for various nucleotides (2.5 ± 0.2 ppm).[18,19]

The proton-decoupled ^{13}C NMR spectra of the cis/trans isomeric 5-substituted 2-pentadecyl-1,3-dioxanes **6**–**8** (Table III) show rather similar chemical shifts[20] for the C-2 signals as well as for the C-4,6 signals of cis/trans isomeric pairs. In contrast, the C-5 signals of *cis*-**6** through *cis*-**8** are shifted downfield by 4.0–4.3 ppm relative to those of the respective trans isomers. The close similarity of C-2 chemical shifts in the spectra of the substituted dioxanes is consistent with the concept of identical 2-alkyl orientation in the cis and trans isomers **5**–**8**, and on the basis of the axial H-2 proton couplings observed by ^1H NMR (Table I), 2-alkyl orientation must be equatorial with respect to the ring. Furthermore, chemical equivalence of carbons 4 and 6, which is manifested by occurrence of identical signals for C-4,6 in all dioxane spectra, would support a strictly symmetrical chair conformation[5] for isomers **5**–**8**. Such symmetry is also borne out by the three-bond carbon–phosphorus couplings as discussed below. Finally, the considerable differences in C-5 chemical shifts in the spectra of cis/trans isomeric pairs substantiates that isomerism occurs about carbon 5 of the 2,5-substituted 1,3-dioxane systems.

Isomerism about C-5 in dioxanes **5**–**8**, i.e., equatorial orientation of the 5-substituent in the trans isomers and axial orientation with resulting 5-substituent/ring interactions in the cis isomers, is consistent with the ^{13}C NMR data which show deshielding and corresponding downfield shifts in the spectra of the cis relative to the trans isomers for C-5 (2.8–4.3 ppm), C-4,6 (0.1–0.2 ppm), the first methylene carbon of the aliphatic chain C-1′ (0.5–0.7 ppm), and the aromatic carbons 2′ and 6′ of diphenyl phosphates **6** (0.4 ppm; see Table III). The C-5 signals in *cis*-**5** (64.2 ppm) and *trans*-**5** (61.4 ppm) in turn are greatly shifted downfield upon phosphorylation of the hydroxymethine group by 7.4 and 5.9 ppm in *cis*-**6** and *trans*-**6** due to diphenyl phosphate, by 5.1 and 3.9 ppm in *cis*-**7** and *trans*-**7** due to phosphate, and by 5.9 and 4.6 ppm in *cis*-**8** and *trans*-**8** due to dimethyl phosphate. Introduction of phosphoryl moieties at C-5 also exerts a considerable shielding effect (1.7–2.6 ppm) on C-4,6.

The carbon-13 NMR spectra of the diphenyl (**2**, **6**) and dimethyl derivatives (**4**, **8**) of cyclic GAP show characteristic two-bond and three-bond **carbon–phosphorus couplings**; cyclic GAP **3** and **7**, most likely due to polar interactions, produced only broadened signals for the respective phosphorus-coupled carbons (see Tables II and III).

Two-bond carbon–phosphorus couplings ($^2J_{POC}$) involve the *exo*-methylene function in position 4 of dioxolanes **2** and **4** (^2J = 5.7–6.3 Hz) and the ring methine C-5 in dioxanes **6** and **8** (^2J = 5.1–6.0 Hz). $^2J_{POC}$ couplings are also observed for the methyl carbons in **4** and **8** (^2J = 5.7–6.3 Hz), and somewhat larger couplings (^2J = 7.1–7.2 Hz) occur for the phenyl C-1′ in **2** and **6**. The magnitude of these $^2J_{POC}$ couplings is generally in line with those observed for sugar phosphates (^2J = 4.4–5.5 Hz)[21] and phosphorylated nucleosides (^2J = 4.0–5.6 Hz),[18,19,22-24] except for the higher ^2J of phenyl C-1′ in **2** and **6** which, similar to those of certain cyclic nucleotides (7.2 ± 0.2 Hz),[23] appear to be due to the drastically altered steric arrangements involving endocyclic carbons.

Three-bond carbon–phosphorus couplings ($^3J_{POCC}$) involve the ring methine C-4 of 2,4-disubstituted dioxolanes **2** and **4** (^3J = 6.7–8.4 Hz) and the ring methylenes C-4,6 of 2,5-disubstituted dioxanes **6** and **8** (^3J = 4.6–6.0 Hz). $^{31}POC^{13}C$ couplings are also observed for the aromatic C-2′,6′ of the diphenyl phosphates **2** and **6** (^3J = 4.8 Hz; see Tables II and III). Three-bond carbon–phosphorus couplings are known to vary widely (1–10 Hz).[18,19,21-24] They are sensitive to dihedral angles, as are $^{31}POC^1H$[25] or vicinal proton couplings and, as the latter, they respond to angular changes in a fashion that is approximated by the Karplus relationship.[23,26] The $^3J_{POCC}$ values measured for the cyclic GAP isomers serve as excellent examples of the dihedral angle dependence of $^{31}POC^{13}C$ couplings. The ^3J values of C-4 in both the phenyl and methyl *trans*-dioxolanes **2** (7.7 Hz) and **4** (7.6 Hz) are rather similar and of a magnitude (≳8 Hz) that would indicate a similar ro-

(18) H. H. Mantsch and I. C. P. Smith, *Biochem. Biophys. Res. Commun.*, **46**, 808 (1972).
(19) I. C. P. Smith, H. J. Jennings, and R. Deslauriers, *Acc. Chem. Res.*, **8**, 306 (1975).
(20) The more substantial shift difference (0.7 ppm) between the C-2 signals of *cis*-**5** and *trans*-**5** (Table III) can be attributed to the effect of the 5-hydroxy function on the dioxane system through interaction with the ring oxygens in the cis isomers. After acetylation[5] of the 5-hydroxy groups of *cis*-**5** and *trans*-**5**, the C-2 signals of *both* isomers appeared at 102.4 ppm. Acetylated *cis*-**5** gave a signal for C-5 at 66.2 ppm and for C-4,6 at 68.7 ppm; acetylated *trans*-**5** showed C-5 at 62.9 ppm and C-4,6 at 68.3 ppm.
(21) A. S. Serianni, J. Pierce and R. Barker, *Biochemistry*, **18**, 1192 (1979).
(22) D. E. Dorman and J. D. Roberts, *Proc. Natl. Acad. Sci. U.S.A.*, **65**, 19 (1970).
(23) (a) R. D. Lapper, H. H. Mantsch, and I. C. P. Smith, *J. Am. Chem. Soc.*, **94**, 6243 (1972); (b) *ibid.*, **95**, 2878 (1973); (c) R. D. Lapper and I. C. P. Smith, *ibid.*, **95**, 2880 (1973).
(24) J. L. Alderfer and P. O. P. Ts'o, *Biochemistry*, **16**, 2410 (1977).
(25) L. D. Hall and R. B. Malcolm, *Chem. Ind. (London)*, 92 (1968).
(26) (a) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); (b) *J. Am. Chem. Soc.*, **85**, 2870 (1963).

tamer population as well as a strong likelihood of trans orientation along POCC in phenyl *and* methyl phosphates *trans*-**2** and *trans*-**4** (Table II). The $^3J_{POCC}$ values of C-4 in *cis*-**2** (8.4 Hz) and in *cis*-**4** (6.7 Hz) are also indicative of a high trans rotamer population. However, the higher coupling in the case of *cis*-**2** appears to support the concept that the bulkier phenyl group in **2** would somewhat limit free rotation and would tend to hold the phenyl phosphate function in a more trans orientation, whereas the smaller methyl group of **4** would cause less interference and would thus permit a higher degree of free rotation and a less homogeneous rotamer population. The validity of this concept is borne out by molecular model experimentation. In contrast, the generally smaller (4.6–6.0 Hz) $^3J_{POCC}$ values of C-4,6 in both cis- and trans-2,5-disubstituted dioxanes **6** and **8** (Table III) are due to the fact that ring carbons 4 and 6 are less likely to assume a trans orientation with respect to phosphate because of ring geometry. Moreover, the greater $^3J_{POCC}$ couplings for *trans*-**6** (6.0 Hz) and *trans*-**8** (5.9 Hz) relative to the couplings of *cis*-**6** (4.8 Hz) and *cis*-**8** (4.6 Hz) would reflect a relatively greater contribution of trans orientation in the trans isomers. This difference could be rationalized by a less homogeneous rotamer population in the trans-disubstituted dioxanes possibly due to greater interference of the phosphate function with H-4,6. Models also confirm that phosphate interference would be largely independent of methyl or phenyl substitution in the dioxane systems.

Experimental Section

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian CFT-20 pulse Fourier-transform instrument equipped for ^{13}C (20 MHz) and ^1H (79.54 MHz) observation. Spectra were measured with 8K data points at a spectral width of 4000 Hz for ^{13}C and with 4K data points at a width of 1000 Hz for ^1H, each at ambient temperature (38 ± 1 and 35 ± 1 °C, respectively). Deuteriochloroform served as solvent and for field-frequency locking purposes, except in the case of phosphates **3** and **7** for which $CDCl_3$–CD_3OD–D_2O, 5:4:1 (v/v/v), was used. ^{13}C data were determined under proton-decoupled conditions; chemical shifts (δ) are expressed in parts per million (ppm) downfield from internal Me_4Si ($\delta = 0.0$). Mass spectra were measured on an LKB 9000 instrument, and samples were inserted through the direct inlet. The ionization potential was 70 eV; source and inlet temperatures were 230 and 190 °C, respectively. Ion intensities are expressed as percentages relative to the most prominent mass peak. Melting points were determined on a Kofler hot stage and are corrected. Elemental analyses were carried out by M-H-W Laboratories, Phoenix, AZ; phosphorus was determined by the modified Bartlett procedure.[27]

Cyclic glycerol acetal phosphoric acid diphenyl esters 2 and **6** were synthesized by phosphorylation of isomerically pure cyclic glycerol acetals *cis*-**1**, *trans*-**1**, *cis*-**5**, and *trans*-**5**[5,6] with diphenyl phosphorochloridate (Aldrich Chemical Co., Milwaukee, WI). A representative procedure is given for the synthesis of *cis*-**2**.

2-Pentadecyl-4-((diphenylphospho)methyl)-1,3-dioxolanes (2). *cis*-2: Cyclic glycerol acetal *cis*-**1** (0.31 g, 1.0 mmol) and dry pyridine (25 mL) were placed in a three-necked flask under nitrogen; diphenyl phosphorochloridate (0.38 g, 1.4 mmol) was added slowly to the solution, and stirring was continued overnight at room temperature. The mixture was evaporated to dryness in vacuo at ambient temperature, the residue was dissolved in Et_2O, and the extract was washed with water (2–3 times) and brought to dryness. Purification by preparative TLC[8] (50:50 hexane–Et_2O, v/v; R_f 0.23) and extraction from the adsorbent with moist Et_2O yielded *cis*-**2** (0.38 g, 69%): mp 36–37 °C; R_f 0.36 (50:50 benzene–Et_2O, v/v); mass spectrum, *m/e* 546 (2.2, M^+), 335 (100, M – $C_{15}H_{31}$), 307 (1.9, M – $C_{15}H_{31}CO$), 251 (22.5, $PO_4H_2Ph_2$), 103 (39.1, M – $C_{15}H_{31}$ – PO_3Ph_2). Anal. Calcd for $C_{31}H_{47}O_6P$: C, 68.11; H, 8.66; P, 5.67. Found: C, 68.47; H, 9.00; P, 5.38. ***trans*-2**: yield after TLC[8] purification (50:50 hexane–Et_2O, v/v; R_f 0.26) 90%: mp 59.5–60.5 °C; R_f 0.57 (50:50 benzene–Et_2O, v/v); mass spectrum, *m/e* 546 (11.1, M^+), 335 (100, M – $C_{15}H_{31}$), 307 (1.4, M – $C_{15}H_{31}CO$), 251 (10.2, $PO_4H_2Ph_2$), 103 (12.1, M – $C_{15}H_{31}$ – PO_3Ph_2). Anal. Calcd for $C_{31}H_{47}O_6P$: C, 68.11; H, 8.66; P, 5.67. Found: C, 68.44; H, 8.67; P, 5.63.

2-Pentadecyl-4-(diphenylphospho)-1,3-dioxanes (6). *cis*-6: yield after TLC[8] purification (50:50 hexane–Et_2O, v/v; R_f 0.21) 68%: mp 64–66 °C; R_f 0.73 (50:50 benzene–Et_2O, v/v); mass spectrum, *m/e* 546 (2.1, M^+), 335 (100, M – $C_{15}H_{31}$), 307 (1.6, M – $C_{15}H_{31}CO$), 251 (37.4, $PO_4H_2Ph_2$), 103 (1.0, M – $C_{15}H_{31}$ – PO_3Ph_2). Anal. Calcd for $C_{31}H_{47}O_6P$: C, 68.11; H, 8.66; P, 5.67. Found: C, 68.18; H, 8.85; P, 5.74. ***trans*-6**: yield after TLC[8] purification (50:50 hexane–Et_2O, v/v; R_f 0.68) 80%; mp 50–52 °C; R_f 0.93 (50:50 benzene–Et_2O, v/v); mass spectrum, *m/e* 546 (14.7, M^+), 335 (100, M – $C_{15}H_{31}$), 307 (1.7, M – $C_{15}H_{31}CO$), 251 (54.8, $PO_4H_2Ph_2$), 103 (1.6, M – $C_{15}H_{31}$ – PO_3Ph_2). Anal. Calcd for $C_{31}H_{47}O_6P$: C, 68.11; H, 8.66; P, 5.67. Found: C, 68.53; H, 8.81; P, 5.44.

Cyclic glycerol acetal phosphoric acids 3 and **7** were prepared by hydrogenolysis of the respective diphenyl esters *cis*-**2**, *trans*-**2**, *cis*-**6** and *trans*-**6**. A representative procedure is given for the synthesis of *cis*-**3**.

2-Pentadecyl-1,3-dioxolane-4-methylphosphoric Acids (3). *cis*-3: Diphenyl ester *cis*-**2** (0.11 g, 0.2 mmol) dissolved in absolute EtOH (5 mL) and a few milligrams of PtO_2 (Adam's Black) were hydrogenated under 40 lb of H_2 for 3 h. The catalyst was filtered off and washed with EtOH and solvent was removed at 30 °C under reduced pressure. Purification by preparative TLC[8] (application, developing, and elution solvent, 50:40:10 $CHCl_3$–MeOH–H_2O, v/v/v; R_f 0.10–0.25, arrowheads) yielded cyclic GAP *cis*-**3** (0.06 g, 76%);[10] mp, sinters at 119 °C, coalesces at 140 °C; mass spectrum, *m/e* 394 (<1, M^+), 313 (1.4, M – PO_3H_2), 103 (100, M – $C_{15}H_{31}$ – PO_3H). Anal. Calcd for $C_{19}H_{39}O_6P$: C, 57.85; H, 9.97; P, 7.86. Found: C, 57.01; H, 9.85; P, 7.78. ***trans*-3**: yield after TLC[8] purification (50:40:10 $CHCl_3$–MeOH–H_2O, v/v/v; R_f 0.10–0.25, arrowheads) 81%; mp 66–67 °C; mass spectrum, *m/e* 394 (<1, M^+), 313 (2.3, M – PO_3H_2), 103 (100, M – $C_{15}H_{31}$ – PO_3H). Anal. Calcd for $C_{19}H_{39}O_6P$: C, 57.85; H, 9.97; P, 7.86. Found: C, 58.04; H, 10.14; P, 7.75.

2-Pentadecyl-1,3-dioxane-4-phosphoric Acids (7). *cis*-7: yield after TLC[8] purification (50:40:10 $CHCl_3$–MeOH–H_2O, v/v/v; R_f 0.15–0.30, arrowheads) 88%; mp 105–108 °C; mass spectrum, *m/e* 394 (<1, M^+), 313 (1.9, M – PO_3H_2), 103 (100, M – $C_{15}H_{31}$ – PO_3H). Anal. Calcd for $C_{19}H_{39}O_6P$: C, 57.85; H, 9.97; P, 7.86. Found: C, 57.78; H, 10.02; P, 7.64. ***trans*-7**: yield after TLC[8] purification (50:40:10 $CHCl_3$–MeOH–H_2O, v/v/v; R_f 0.25–0.40, arrowheads) 94%; mp 100–104 °C; mass spectrum, *m/e* 394 (<1, M^+), 313 (1.4, M – PO_3H_2), 103 (100, M – $C_{15}H_{31}$ – PO_3H). Anal. Calcd for $C_{19}H_{39}O_6P$: C, 57.85; H, 9.97; P, 7.86. Found: C, 58.04; H, 10.14; P, 7.64.

Cyclic glycerol acetal phosphoric acid dimethyl esters 4 and **8** were prepared for further characterization of the individual free phosphates **3** and **7** and to assure configurational stability throughout the synthesis by comparison with diphenyl esters **2** and **6**. For this purpose, cyclic glycerol acetal phosphates *cis*-**3**, *trans*-**3**, *cis*-**7**, and *trans*-**7** were each methylated[11] with diazomethane (caution).[28] A representative procedure is given for the preparation of *cis*-**4**.

2-Pentadecyl-4-((dimethylphospho)methyl)-1,3-dioxolanes (4). *cis*-4: Cyclic GAP *cis*-**3** dried in vacuo (0.39 g, 1.0 mmol) was taken up in anhydrous benzene, lyophilized to a white powder, and immediately dissolved under nitrogen in anhydrous Et_2O containing 5% MeOH. Methylation[28] at room temperature was continued until excess diazomethane became evident by yellow discoloration. The solvent was removed under nitrogen; the sample was taken up in chloroform, purified by preparative TLC[8] (10:90 hexane-Et_2O, v/v; R_f 0.14), and eluted from the adsorbent with moist Et_2O to yield dimethyl phosphate *cis*-**4** (0.27 g, 65% yield): mp 38–39 °C; mass spectrum, *m/e* 422 (1.1, M^+), 211 (100,

(27) (a) G. R. Bartlett, *J. Biol. Chem.*, **234**, 466 (1959); (b) G. V. Marinetti, *J. Lipid Res.*, **3**, 1 (1962).

(28) **Caution**: Diazomethane is highly toxic and explosive. Ground joints and scratched glassware are to be avoided. (a) *Org. React.*, **8**, 392 (1954); (b) Th. J. de Boer and H. J. Backer, "Organic Syntheses", Collect. Vol. 4, Wiley, New York, 1963, p 250.

M - $C_{15}H_{31}$), 183 (8.3, M - $C_{15}H_{31}CO$), 127 (61.8, $PO_4H_2Me_2$), 109 (22.1, PO_3Me_2). Anal. Calcd for $C_{21}H_{43}O_6P$: C, 59.69; H, 10.26; P, 7.33. Found: C, 60.02; H, 10.54; P, 7.37. ***trans*-4**: yield after TLC[8] purification (10:90 hexane-Et_2O, v/v; R_f 0.15) 73%; mp 37-38 °C; mass spectrum, *m/e* 422 (<1, M^+), 211 (100, M - $C_{15}H_{31}$), 183 (5.1, M - $C_{15}H_{31}CO$), 127 (62.0, $PO_4H_2Me_2$), 109 (25.2, PO_3Me_2). Anal. Calcd for $C_{21}H_{43}O_6P$: C, 59.69; H, 10.26; P, 7.33. Found: C, 59.90; H, 10.47; P, 7.11.

2-Pentadecyl-4-(dimethylphospho)-1,3-dioxanes (8). *cis*-8: yield after TLC[8] purification (10:90 hexane-Et_2O, v/v; R_f 0.08) 77%; mp 53-54 °C; mass spectrum, *m/e* 422 (<1, M^+), 211 (100, M - $C_{15}H_{31}$), 183 (2.9, M - $C_{15}H_{31}CO$), 127 (75.7, $PO_4H_2Me_2$), 109 (11.0, PO_3Me_2). Anal. Calcd for $C_{21}H_{43}O_6P$: C, 59.69; H, 10.26; P, 7.33. Found: C, 59.91; H, 10.55; P, 7.32. ***trans*-8**: yield after TLC[8] purification (10:90 hexane-Et_2O, v/v; R_f 0.27) 59%; mp 50.5-51.5 °C; mass spectrum, *m/e* 422 (<1, M^+), 211 (89.5, M - $C_{15}H_{31}$), 183 (3.1, M - $C_{15}H_{31}CO$), 127 (100, $PO_4H_2Me_2$), 109 (12.5, PO_3Me_2). Anal. Calcd for $C_{21}H_{43}O_6P$: C, 59.69; H, 10.26; P, 7.33. Found: C, 60.04; H, 10.46; P, 7.27.

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Registry No. *cis*-1, 30889-28-2; *trans*-1, 30889-31-7; *cis*-2, 73116-77-5; *trans*-2, 73116-78-6; *cis*-3, 73116-79-7; *trans*-3, 73116-80-0; *cis*-4, 73116-81-1; *trans*-4, 73116-82-2; *cis*-5, 30889-22-6; *trans*-5, 30889-25-9; *cis*-6, 73116-83-3; *trans*-6, 73116-84-4; *cis*-7, 73116-85-5; *trans*-7, 73116-86-6; *cis*-8, 73116-87-7; *trans*-8, 73116-88-8; diphenylphosphorochloridate, 2524-64-3.

Marine Alkaloids. 2. Bromo Alkaloids from the Marine Bryozoan *Flustra foliacea*. Isolation and Structure Elucidation

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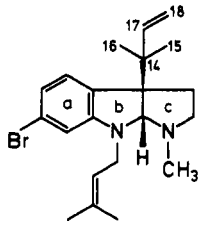
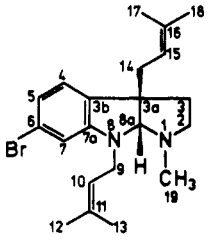
Flustramines A and B, two bromo-substituted alkaloids, were isolated from the marine bryozoan *Flustra foliacea*. The isolation procedure and detailed assignment of all ^{13}C and ^1H NMR data are reported. Nuclear Overhauser enhancement difference spectroscopy reveals the pyrrolidine rings to be cis fused, in close analogy with physostigmine.

During the last decade, the biology of the phylum Bryozoa (syn. Polyzoa, Ectoprocta, or moss animals) has received a great deal of attention,[1] and much data have accumulated about the chemistry of marine natural products.[2] However, despite these facts, chemical investigations of Bryozoa seem to be virtually nonexistent. This is the more curious since the phylum is represented by species abundantly available in fresh water as well as in the marine environment, where they greatly add to the problem of "fouling".[1,3]

A study of the chemical ecology of the marine bryozoan *Flustra foliacea* (L.) revealed this species to contain an allelochemical mixture of monoterpenes,[4] as well as a mixture of brominated alkaloids.[5] The literature on marine alkaloids is very scarce; moreover, the few bromo-substituted members of this class seem to bear no resemblance to patterns established from terrestrial sources. In this paper we wish to report the detailed isolation procedure and structural assignments of flustramines A (1) and B (2),[6] two brominated alkaloids refer-



1. FLUSTRAMINE A



2. FLUSTRAMINE B

rable to the basic physostigmine skeleton known from the minor group of alkaloids from Calabar bean (*Physostigma venenosum* Balf. of family Leguminosae).

Results and Discussion

Isolation. Repeated column chromatography (silica gel) of a petroleum ether extract of freeze-dried bryozoans left 25 mg of each alkaloid as colorless oils. High-resolution mass spectrometry revealed the compounds to be isomers with the elemental composition $C_{21}H_{29}BrN_2$. As discussed below, spectral analysis made it apparent that the species differ only in the structure of one of the side chains.

Mass Spectra. Fragment ions at *m/e* 210/208 appearing in the spectra of flustramines A and B were found to have the elemental composition C_9H_7NBr. We attribute

(1) J. S. Ryland, "Bryozoans", Hutchinson University Library, London, 1970; R. M. Woollacott and R. L. Zimmer, Eds., "Biology of Bryozoans", Academic Press, New York, San Francisco, London, 1977.
(2) P. J. Scheuer, Ed., "Marine Natural Products, Chemical and Biological Perspectives", Vol. II, Academic Press, New York, San Francisco, London, 1978.
(3) R. D. Barnes, "Invertebrate Zoology", 2nd ed., W. B. Saunders, Philadelphia, 1968, pp 588-99.
(4) C. Christophersen and J. S. Carlé, *Naturwissenschaften*, **65**, 440 (1978).
(5) J. S. Carlé and C. Christophersen, *J. Am. Chem. Soc.*, **101**, 4012 (1979).
(6) The figures of flustramines A and B do not depict the absolute configuration of the molecules.

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