of ionization used to calculate the pK' values at 10° were the following: +0.76 kcal/mol (alanine) for carboxylic acids,[52] +9.13 kcal/mol (SMC) for ammonium groups,[3f] and +6.9 kcal/mol (2-mercaptoacetic acid) for thiol groups.[53,54]

(52) J. T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. I, Academic Press, New York, N. Y., 1958.

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New Synthesis and Absolute Configuration of Tetrahydroisoquinoline Cactus Alkaloids

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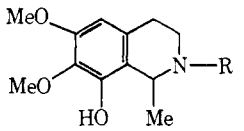
Abstract: The 7,8-methylenedioxy-substituted alkaloids, (−)-anhalonine (**3**) and (−)-lophophorine (**4**), have been prepared by a new and facile synthesis which utilized the 7,8-diphenol **6** as the key intermediate. X-Ray crystallographic studies of **3**·HBr and **11**·HBr established that both **3** and **4** as well as the related alkaloid (+)-*O*-methylanhalonidine (**11**) possess the *S* configuration.

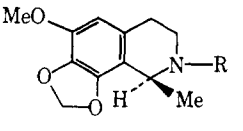
In connection with the structure elucidation of the four major tetrahydroisoquinolines found in the peyote cactus, Späth and coworkers synthesized (±)-anhalonidine[1] (**1**), (±)-pellotine[1] (**2**), (−)-anhalonine[2,3] (**3**), and (−)-lophophorine[2,3] (**4**). Since this classic study, alternate methods have been developed for the preparation of the 6,7-dimethoxy-8-hydroxy-substituted alkaloids **1** and **2**[4] but not for the 6-methoxy-7,8-methylenedioxy-substituted alkaloids **3** and **4**. We now describe a novel and simplified synthesis of **3** and **4** which is based on the partial O-demethylation of the 6,7,8-trimethoxy-substituted tetrahydroisoquinoline **5** to provide the corresponding (±)-6-methoxy-7,8-diphenol **6** as the key intermediate.[5]

Treatment of (±)-*O*-methylanhalonidine[6] (**5**) with 20% hydrochloric acid under controlled conditions afforded the 6-methoxy-7,8-diphenol **6**[5] which was reacted with ethyl chloroformate to yield the carbamate **7**. Cupric oxide catalyzed condensation of **7** with dibromomethane in dimethylformamide[7] provided the methylenedioxy derivative **8** which was either converted into (±)-anhalonine (**9**) by base-catalyzed hydrolysis or into (±)-lophophorine (**10**) by lithium aluminum hydride reduction. Alternatively, **10** was also obtained by the reductive condensation of **9** with formaldehyde in the presence of Raney nickel catalyst. Resolution of **9** with (−)-tartaric acid according to the procedure of Späth and Kesztler[3] afforded the alkaloid (−)-anhalonine (**3**) which was transformed by N-methylation into natural lophophorine (**4**).

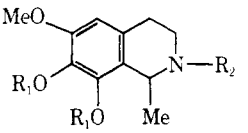
Although both **3** and **4** have been assigned the *S* configuration by the method of optical rotatory shifts,[8] this approach appeared unreliable for the related tetrahydroisoquinoline (+)-*O*-methylanhalonidine (**11**). The latter alkaloid, a minor constituent in the peyote cactus, was prepared from **5** by resolution with (+)-tartaric acid[9] and exhibited an anomalous optical behavior in solvents of different polarity. Unlike **3** and **4**, **11** showed the same molecular rotation in both chloroform and 1 *N* hydrochloric acid. In view of this, it was deemed advisable to confirm the absolute configurations of **3** and **4** and establish that of **11** by X-ray crystallography. For this purpose, **3**·HBr and **11**·HBr were chosen for study.



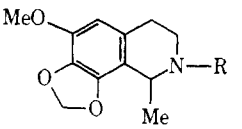
1, R = H
2, R = Me



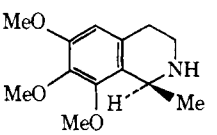
3, R = H
4, R = Me



5, R_1 = Me; R_2 = H
6, R_1 = R_2 = H
7, R_1 = H; R_2 = CO_2Et



8, R = CO_2Et
9, R = H
10, R = Me



11

Pertinent results of the crystallographic studies are shown in Figures 1, 2, and 3. Bond lengths and angles involving the nonhydrogen atoms of **3**·HBr[10] and **11**·

(1) E. Späth, *Monatsh. Chem.*, 43, 477 (1922); E. Späth and F. Becke, *Chem. Ber.*, 67, 266 (1934).
(2) E. Späth and J. Gange, *Monatsh. Chem.*, 44, 103 (1923).
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(5) A. Brossi and S. Teitel, *Chem. Commun.*, 1296 (1970).
(6) S. Karady, *J. Org. Chem.*, 27, 3720 (1962).
(7) M. Tomita and Y. Aoyagi, *Chem. Pharm. Bull.*, 16, 523 (1968).
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(9) E. Späth and J. Bruck, *Chem. Ber.*, 72, 334 (1939).
(10) The bond lengths have been averaged over the thermal motion with one atom assumed to ride on the other; see W. R. Busing and H. A. Levy, *Acta Crystallogr.*, 17, 142 (1964).

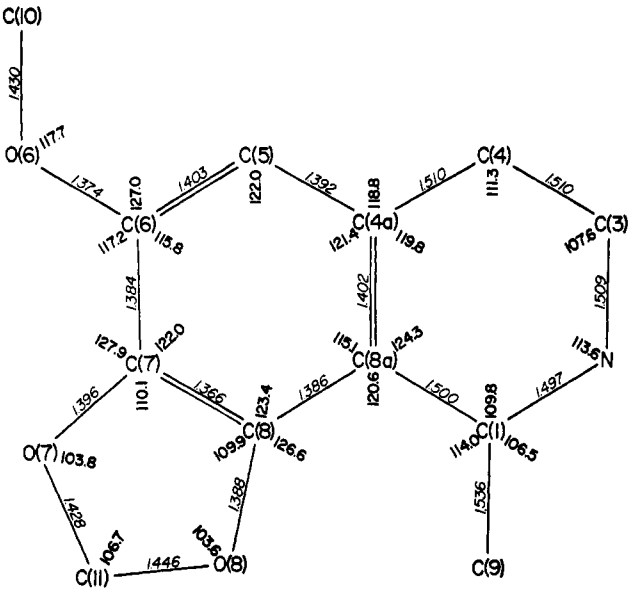

Figure 1. Bond lengths and angles in **3**·HBr.[10]

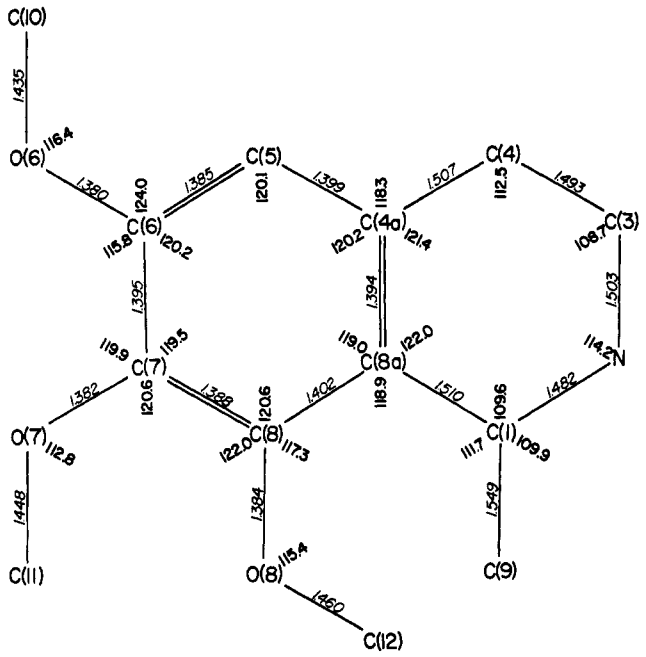

Figure 2. Bond length and angles in **11**·HBr.[10]

HBr are shown in Figures 1 and 2, respectively. Estimated standard deviations of the bond lengths and angles are 0.007 Å and 0.4° for **3**·HBr and 0.005 Å and 0.3° for **11**·HBr. The average C–H distance is 0.98 Å for both salts.

It is evident from the stereodrawings (Figure 3) that both **3** and **11** possess the *S* configuration. Further, careful examination of the drawings will reveal that the sense of the distortion of the heterocyclic ring from the plane of the phenyl ring in **3**·HBr is opposite to that in **11**·HBr. That is, if N and C(3) of **3**·HBr are considered to be above and below the plane of the phenyl ring, respectively, then N and C(3) in **11**·HBr are below and above the analogous plane, respectively. As a result, the 1-methyl [*i.e.*, $C(9)H_3$] is axial in **11**·HBr but is pseudoequatorial in **3**·HBr. The O(8)–C(9) distances are 3.19 Å in **11**·HBr and 3.10 Å in **3**·HBr. Distances of the atoms from the planes of the phenyl rings are listed in Table I for the two compounds.

Table I. Distances in Ångströms from the Best Planes through the Phenyl Planes [C(4a), C(5), C(6), C(7), C(8), C(8a)] in **3**·HBr and **11**·HBr.

Atom		Distance	
3·HBr	**11**·HBr	**3**·HBr	**11**·HBr
	C(4a)	−0.02	−0.02
	C(5)	−0.01	0.02
	C(6)	0.02	0.01
	C(7)	−0.01	−0.03
	C(8)	−0.02	0.02
	C(8a)	0.03	0.00
	O(6)	0.08	0.04
	O(7)	−0.02	−0.09
	O(8)	−0.08	0.03
	C(10)	0.09	0.22
	C(11)		−1.38
	C(12)		1.09
C(11)		−0.42	
	C(4)	−0.10	−0.15
	C(3)	−0.59	0.23
	N	0.28	−0.43
	C(1)	0.11	0.04
	C(9)	1.28	1.45

In each compound, the three oxygens all lie within 0.1 Å of the plane of the phenyl ring. The dihedral angle between the 6-methoxy plane (*i.e.*, the plane formed by C(10), O(6), C(6)) and the phenyl ring is 2.4° in **3**·HBr and 7.9° in **11**·HBr whereas the dihedral angles between the 7-methoxy and 8-methoxy planes and the phenyl ring in **11**·HBr are 72 and 54°, respectively. In this connection, it is of interest to note that the 7- and 8-methoxyls in **5** are cleaved in preference to the 6-methoxyl under controlled reaction conditions.[5] This may be explained by the fact that the methyl groups associated with the 7- and 8-methoxyls in **11**·HBr are out of the plane of the aromatic ring.

The methyls of the 6-methoxy groups in **3**·HBr and **11**·HBr position themselves in a manner similar to that which has recently been described for the methyls in the $C{=}COCH_3$ groups in the cyclic lactam obtained on irradiation of *N*-chloroacetylmescaline.[11] Thus, in **3**·HBr and **11**·HBr, one H of each $C(10)H_3$ (Figure 3) lies within 0.05 Å of the C_{Me}–O–C_{ring} plane and is directed away from the ring. The tendency of methoxyls attached to a phenyl ring to lie in the plane of the ring whenever possible is illustrated by the 6-methoxyls of **3**·HBr and **11**·HBr and by the two outside methoxyls of the trimethoxybenzoic acid ester moiety of reserpine.[12] A comparison of the C_{sp^2}–O and O–C_{Me} bond lengths for these methoxy groups[13] does not reveal any significant differences in these values for the OCH_3 groups in which the C_{Me} lies in the plane of the C_{sp^2} σ bonds and those in which the C_{Me} is out of the plane. There is, however, a significant difference (3.6°) in the bond angles for the 6- and 7-methoxys of **11**·HBr, where the methoxy in the plane

(11) O. Yonemitsu, H. Nakai, Y. Kanoaka, I. L. Karle, and B. Witkop, *J. Amer. Chem. Soc.*, **92**, 5691 (1970).
(12) I. L. Karle and J. Karle, *Acta Crystallogr., Sect. B*, **24**, 81 (1968).
(13) For comparison with the corresponding values reported for the cyclic lactam[11] and reserpine,[12] the distances, uncorrected for thermal motion, are 1.357, 1.369, 1.371, 1.372 Å for C_{sp^2}–O and 1.430, 1.429, 1.435, 1.443 Å for O–C_{Me} at the 6 position in **3**·HBr and at the 6, 7, and 8 positions in **11**·HBr, respectively.

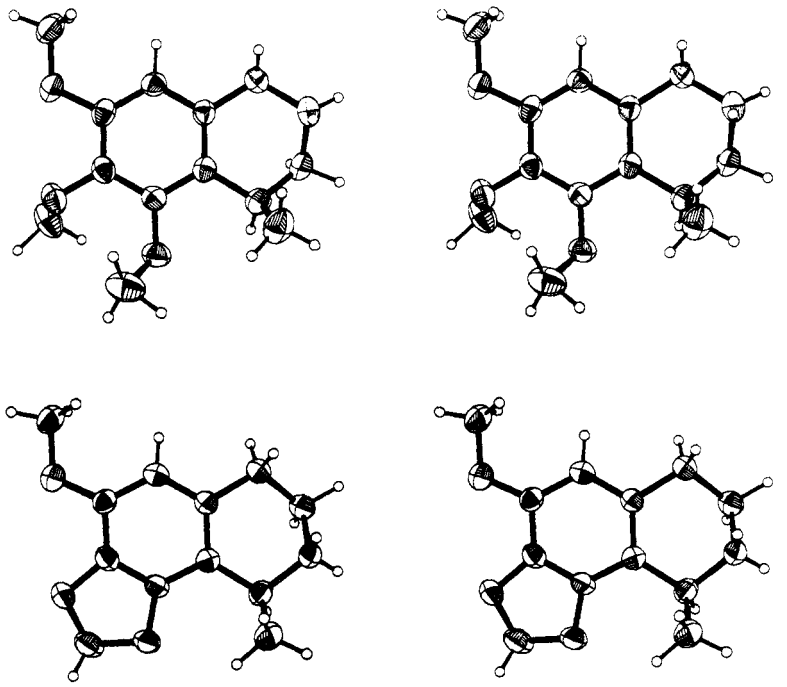

Figure 3. Stereodrawings illustrating the absolute configurations of **11** (upper) and **3** (lower) as determined from the X-ray analyses of **11**·HBr and **3**·HBr. The ellipsoids represent the thermal motions of each atom at the 50% probability level. The drawings were prepared by a computer program written by C. K. Johnson of the Oak Ridge National Laboratory. Stereoviewers suitable for viewing the above may be obtained from Abrams Instrument Co., Lansing, Mich. (Model C F 8), Wards Natural Science Establishment, Inc., Rochester, N. Y. (Model 25 W 2951), and other suppliers.

has the greater angle, presumably because of its close nonbonded approach to C(5) (2.87 Å) (the 8-methoxy angle is not considered because of the close proximity of the 1-methyl). A similar increase in the C_{ring}–O–C_{Me} angles for the OCH_3 groups in the phenyl plane is seen in reserpine.

Based on the similarity of the ORD and CD spectra of **3**, **4**, and **11** (see Experimental Section), it is certain that **4** also possesses the *S* configuration. Thus, the initial assignments for **3** and **4** are verified and the absolute configurations of the three cactus alkaloids (−)-anhalonine (**3**), (−)-lophophorine (**4**), and (+)-O-methylanhalonidine (**11**) are unequivocally established.

Experimental Section

All melting points (corrected) were taken in open capillary tubes with a Thomas-Hoover melting apparatus. Nuclear magnetic resonance (nmr) spectra were obtained with a Varian A-60 or HA-100 spectrometer, and peak positions are given in parts per million downfield from tetramethylsilane as an internal standard. The abbreviations s, d, t, q, and m refer to singlet, doublet, triplet, quartet, and multiplet; the coupling constant (*J*) is measured in hertz. The ultraviolet spectra were measured in ethanol with a Cary recording spectrophotometer Model 14M. Optical rotations were obtained with a Perkin-Elmer polarimeter Model 141. Rotatory dispersion curves were determined at 23° with a Durrum-Jasco spectrophotomer Model 5 using 1-cm, 0.1-cm, or 0.1-mm cells. Circular dichroism curves were measured on the same instrument and are expressed in molecular ellipticity units [θ]. The mass spectra were taken with a CEF 21-110 mass spectrometer at 70 eV using a direct insertion probe. Extracts of products in organic solvents were washed with water and dried over sodium sulfate prior to evaporation.

(±)-7,8-Dihydroxy-6-methoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline Hydrochloride (6·HCl). A solution of 9 g (0.033 mole) of (±)-*O*-methylanhalonidine hydrochloride[6] (**5**·HCl) in 90 ml of 20% hydrochloric acid was stirred and refluxed for 13 hr. After storage at 4° for 3 hr, the crystals were collected, air-dried, and recrystallized from ethanol to give 4.6 g (57%) of **6**·HCl, mp 263–265° (lit.[5] mp 263–265°). *Anal.* Calcd for $C_{11}H_{15}NO_3 \cdot HCl$: C, 53.77; H, 6.56. Found: C, 53.78; H, 6.83.

(±)-2-Carbethoxy-7,8-dihydroxy-6-methoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline (7). A solution of 8.2 g (0.076 mole) of ethyl chloroformate in 50 ml of methylene chloride was added over 2 hr to a vigorously stirred mixture of 17 g (0.069 mole) of **6**·HCl in 100 ml of methylene chloride and 100 ml of water which was maintained at 10–15° and slightly alkaline by the addition of 10% sodium hydroxide as needed. After stirring at room temperature for 2 hr, the organic layer was separated and evaporated, and the residual oil was vacuum distilled to give 16.5 g (85%) of **7**: bp 195–197° (0.05 mm); $n^{25}D$ 1.4470; nmr ($CDCl_3$) δ 1.27 (t, 3, J = 7 Hz, CH_3CH_2), 1.43 (d, 3, J = 7 Hz, CH_3CH), 3.82 (s, 3, CH_3O), 4.20 (q, 2, J = 7 Hz, CH_2O), 5.64 (b, 2, 2 OH), 6.25 (s, 1, aromatic). *Anal.* Calcd for $C_{14}H_{19}NO_5$: C, 59.77; H, 6.81. Found: C, 60.01; H, 6.99.

(±)-2-Carbethoxy-6-methoxy-1-methyl-7,8-methylenedioxy-1,2,3,-4-tetrahydroisoquinoline (8). A mixture of 18.7 g (0.065 mole) of **7**, 34 g (0.2 mole) of dibromomethane, 39 g (0.28 mole) of anhydrous potassium carbonate, and 0.3 g of powdered cupric oxide in 150 ml of freshly distilled dimethylformamide was stirred and refluxed for 7 hr, cooled, and evaporated under reduced pressure. The residue was suspended in 100 ml of water, adjusted to pH 12 with 10% sodium hydroxide, and extracted with three 100-ml portions of methylene chloride. The combined extracts were evaporated, and the residual oil was vacuum distilled to give 14.8 g (76%) of **8**: bp 190–193° (0.07 mm); $n^{25}D$ 1.5380; nmr ($CDCl_3$) δ 1.29 (t, 3, J = 7 Hz, CH_3CH_2), 1.34 (d, 3, J = 7 Hz, CH_3CH), 3.86 (s, 3, CH_3O), 4.16 (q, 2, J = 7 Hz, CH_2O), 5.90–5.98 (AB, 2, J = 1 Hz, OCH_2O), 6.28 (s, 1, aromatic). *Anal.* Calcd for $C_{15}H_{19}NO_5$: C, 61.42; H, 6.53. Found: C, 61.47; H, 6.76.

(±)-Anhalonine Hydrochloride (9·HCl). A solution of 7.3 g (0.025 mole) of **8** in 100 ml of ethanol and 100 ml of 10% sodium hydroxide was refluxed overnight, cooled, and evaporated under reduced pressure. The residue was suspended in 100 ml of water and extracted with three 100-ml portions of ether. The combined extracts were acidified with ethanolic hydrogen chloride and evaporated, and the residue was crystallized from methanol to give 5 g (78%) of **9**·HCl: mp 262–264°; nmr (DMSO-d_6) δ 1.56 (d, 3, J = 6 Hz, CH_3CH), 3.78 (s, 3, CH_3O), 5.96, 6.02 (s, 2, OCH_2O), 6.49 (s, 1, aromatic); uv max 214 mμ (ϵ 47,350), infl 250 (2700), 277 (980), infl 286 (880). *Anal.* Calcd for $C_{12}H_{15}NO_3 \cdot HCl$: C, 55.93; H, 6.26. Found: C, 55.77; H, 6.32.

(±)-**Lophophorine Hydrobromide (10·HBr).** A solution of 5.8 g (0.02 mole) of **8** in 100 ml of tetrahydrofuran was added over 15 min to a stirred and refluxing suspension of 2 g of lithium aluminum hydride in 100 ml of tetrahydrofuran. The mixture, after refluxing for 2 hr, was cooled and the excess hydride was decomposed by cautious addition of ether saturated with water and filtered. The filtrate was evaporated, and the residue was dissolved in ethanolic hydrogen bromide, evaporated, and crystallized from ethanol to give 5 g (80%) of **10·HBr**: mp 221–222°; nmr (DMSO-d_6) δ 1.58 (d, 3, J = 6.5 Hz, CH_3CH), 2.90 (s, 3, CH_3N), 3.81 (s, 3, CH_3O), 4.55 (q, 1, J = 6.5 Hz, CHN), 5.98, 6.04 (s, 2, OCH_2O), 6.52 (s, 1, aromatic), 10.2 (b, 1, N^+H); uv max 211 mμ (ϵ 43,000), infl 250 (2950), 278 (1065), infl 286 (960).

Alternatively, to a solution of 1.97 g (7.7 mmoles) of **9·HCl** in 150 ml of methanol, neutralized with a methanolic solution of sodium methylate, was added 3 ml of 35% formaldehyde and the mixture hydrogenated in the presence of 1 g of Raney nickel at 3 atm and room temperature until the hydrogen uptake had ceased. The mixture was filtered and the filtrate evaporated. The residue was acidified with ethanolic hydrogen bromide, evaporated, and crystallized from ethanol to give 2.2 g (90%) of **10·HBr**. *Anal.* Calcd for $C_{13}H_{17}NO_3 \cdot HBr$: C, 49.38; H, 5.74. Found: C, 49.11; H, 5.84.

(*S*)-(−)-**Anhalonine (3).** Resolution of 8.5 g (0.037 mole) of **9** with (−)-tartaric acid according to the procedure of Späth and Kesztler[3] afforded 6 g of the tartrate salt of **3** [mp 200–201°, $[\alpha]^{25}D$ −33.6° (*c* 1, H_2O)] which was dissolved in water, rendered alkaline with 5% NaOH, and extracted with ethyl acetate. The organic extracts were evaporated and the residue was crystallized from a mixture of ether and petroleum ether (30–60°) to give 2.7 g (63%) of **3** which exhibited the following detailed data: mp 83–84°; $[\alpha]^{25}D$ −54.0° (*c* 1, $CHCl_3$) [lit.[3] mp 85–86°, $[\alpha]^{25}D$ −56.3° (*c* 2.7, $CHCl_3$); lit.[14] mp 84–85°, $[\alpha]^{26}D$ −62° (*c* 0.78, $CHCl_3$)]; $[\alpha]^{25}D$ −62.9° (*c* 1, MeOH), $[\alpha]^{25}D$ −51.0° (*c* 1, 1 *N* HCl); uv and nmr spectra similar to natural anhalonine;[13] ORD (*c* 0.22, MeOH) $[\phi]_{700}$ −94°, $[\phi]_{589}$ −137°, $[\phi]_{288}$ −1130° (tr), $[\phi]_{252}$ −754° (pk), and $[\phi]_{230}$ −6790° (tr); CD (*c* 0.01 *M*, MeOH), $[\theta]_{290}$ 0, $[\theta]_{280}$ −463, $[\theta]_{256}$ 0, $[\theta]_{242}$ +2110, $[\theta]_{234}$ 0, and $[\theta]_{224}$ −6640; mass spectrum *m/e* (rel intensity) 221 (10), 220 (7.2), 206 (100), 192 (3.7), 191 (6), 176 (1), 161 (1.5), 147 (1.1), 133 (1.9), 118 (1.1), 104 (2.1), 103.5 (1.3), 91 (1.6), 77 (1.8), 65 (1.5), 63 (1.2), 51 (1.6), 39 (1). *Anal.* Calcd for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.23; H, 6.79; N, 6.18.

The hydrochloride of **3** exhibited: mp 258–259° [lit.[14] mp 260–261°]; $[\alpha]^{25}D$ −40.0° (*c* 1, 50% EtOH) [lit.[15] $[\alpha]^{25}D$ −40.5° (*c* 1, 50% EtOH)]; and **3·HBr** exhibited: mp 270–271° (from H_2O); $[\alpha]^{25}D$ −30.0° (*c* 1, H_2O). *Anal.* Calcd for $C_{12}H_{15}NO_3 \cdot HBr$: C, 47.80; H, 5.34; N, 4.64. Found: C, 48.00; H, 5.29; N, 4.51.

(*S*)-(−)-**Lophophorine (4).** By the procedure given for **10**, 1.5 g (6.8 mmoles) of **3** was reductively N-methylated to give 1.5 g (82%) of **4·HCl**: mp 236–237° (from ethanol) (lit.[14] mp 233–235.5°); $[\alpha]^{25}D$ −15.6° (*c* 1, H_2O) [lit.[3] $[\alpha]^{25}D$ −16.3° (*c* 4, H_2O)]; mass spectrum *m/e* (rel intensity) 235 (2.5), 234 (3.0), 220 (100), 205 (5.0), 192 (3.8), 175 (1.4), 162 (0.7), 161 (0.6), 160 (0.4), 159 (0.5), 147 (2.0), 131 (0.8), 118 (1.3), 110 (2.6), 109.5 (2.0), 91 (1.6), 77 (1.6), 51 (1.3), 42 (2.0), 36 (2.4).

The free base **4**, obtained from **4·HCl** by neutralization followed by distillation, exhibited: bp 140–145° (0.05 mm) [lit.[3] bp 140–150° (0.02 mm)]; $[\alpha]^{25}D$ −46.8° (*c* 5, $CHCl_3$) [lit.[3] $[\alpha]^{25}D$ −47.3° (*c* 5, $CHCl_3$); lit.[14] $[\alpha]^{25}D$ −62° (*c* 0.78, $CHCl_3$)]; uv and nmr spectra similar to natural lophophorine;[14] ORD (*c* 0.2, MeOH) $[\phi]_{700}$ −72°, $[\phi]_{589}$ −100°, $[\phi]_{287}$ −814° (tr), $[\phi]_{253}$ +175° (pk), and $[\phi]_{230}$ −10,180° (tr); CD (*c* 0.009 *M*, MeOH) $[\theta]_{294}$ 0, $[\theta]_{279}$ −605, $[\theta]_{262}$ 0, $[\theta]_{245}$ +2930, $[\theta]_{235}$ 0, $[\theta]_{223}$ −9310, and $[\theta]_{216}$ 0. *Anal.* Calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.05; H, 7.43; N, 5.77.

(*S*)-(+)-*O*-**Methylanhalonidine (11).** Utilizing the procedure of Späth and Bruck,[9] 23.4 g (0.1 mole) of (±)-*O*-methylanhalonidine[6] (**5**) was resolved with (+)-tartaric acid to afford 12 g (62%) of the tartrate salt of **11**: mp 190–191°; $[\alpha^{25}D$ +27.0° (*c* 1, MeOH). *Anal.* Calcd for $C_{13}H_{19}NO_3 \cdot C_4H_6O_6$: C, 52.71; H, 6.51. Found: C, 52.67; H, 6.86.

The free base **11**, obtained as a colorless, viscous oil by neutralization of the above salt followed by distillation, exhibited: bp 150° (0.07 mm) [lit.[9] bp 140° (0.05 mm)]; $[\alpha]^{25}D$ +11.5° (*c* 1, MeOH); $[\alpha]^{25}D$ +19.7° (*c* 11, MeOH) [lit.[9] $[\alpha]^{16}D$ +20.7° (*c* 11, MeOH)], $[\alpha]^{25}D$ +20.6° (*c* 1, $CHCl_3$); $[\alpha]^{25}D$ + 19.3° (*c* 1, 1 *N* HCl); nmr ($CDCl_3$) δ 1.43 (d, 3, J = 7 Hz, CH_3C), 1.87 (s, 1, NH), 2.50–3.30 (b, 4, CH_2CH_2), 3.87, 3.87, 3.97 (s, 9, 3, CH_3O), 4.28 (q, 1, J = 7 Hz, CH), 6.45 (s, 1, aromatic); uv max 207 mμ (ϵ 40,700), infl 228 (9360), 273 (1220), 280 (1280); ORD (*c* 0.22, MeOH) $[\phi]_{700}$ +14°, $[\phi]_{589}$ +23°, $[\phi]_{288}$ +512° (pk), $[\phi]_{283}$ +380° (tr), $[\phi]_{238}$ +4130° (pk), $[\phi]_{230}$ +2200° (tr); CD (*c* 0.01 *M*, MeOH) $[\phi]_{295}$ 0, $[\phi]_{279}$ +165, $[\phi]_{254}$ +11, $[\phi]_{233}$ +2970, $[\theta]_{223}$ +1210, and $[\theta]_{208}$ +29,730. *Anal.* Calcd for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.62; H, 7.96; N, 5.76.

In the usual manner, the above free base and alcoholic HBr afford, after crystallization from water, **11·HBr**: mp 202–204°; $[\alpha]^{25}D$ +16.4° (*c* 1, MeOH). *Anal.* Calcd for $C_{13}H_{19}NO_3 \cdot HBr$: C, 49.07; H, 6.34. Found: C, 49.09; H, 6.46.

Crystallography. Both **3·HBr** and **11·HBr** crystallize in the orthorhombic crystal system. Pertinent crystal data for these two salts are given in Table II. The intensity data for both compounds were collected by a moving crystal–moving detector method on a Hilger & Watts Model Y290 four-circle diffractometer. Nickel-filtered Cu Kα radiation and pulse height discrimination were used. In each case, the intensities of two complete octants of data were recorded such that Bijvoet pairs of reflections were obtained. A total of 2776 reflections were measured in the two octants for **3·HBr**, of which 460 were unobservedly weak (3506 and 761 for **11·HBr**). All observed data were corrected for absorption.[16] In the least-squares refinement, however, only the observed data from the *hkl* octant were used (1212 reflections for **3·HBr**, 1414 for **11·HBr**). The crystals which were used for data collection had the approximate dimensions: **3·HBr**, 0.06 × 0.44 × 0.07 mm; **11·HBr**, 0.22 × 0.10 × 0.40 mm.

Table II. Crystal Data

	3·HBr	11·HBr
Formula weight	302.18	318.22
Space group	$P2_12_12_1$	$C222_1$
a, Å	22.886 (12)	9.382 (3)
b, Å	5.163 (4)	18.550 (5)
c, Å	10.863 (7)	17.509 (5)
V, Å^3	1283.6	2883.2
d_{obsd}, g cm^{-3}	1.59	1.45
d_{calcd} g cm^{-3}	1.56	1.47
Z	4	8
μ, cm^{-1}	48.2	42.8

The function minimized in the least-squares refinement was $\Sigma w||F_o| - |F_c||^2$ where $w = 1/(a + bF_o + F_o^2)$ (a, b, c were 7.3, 0.6, 0.008 for **3·HBr** and 8.0, 1.2, 0.014 for **11·HBr**, respectively). Standard atomic scattering curves were used for Br^-, C, N, O,[17] and H.[18] The Br^- curve was corrected for the real and imaginary parts of the anomalous scattering.[19] The refinement calculations were made with a local modification of the program ORFLS.[20]

The structure of **3·HBr** was solved by the heavy atom method. Initial refinement was by full-matrix least squares in which the atoms were assigned isotropic temperature factors. The atoms were then assigned anisotropic thermal parameters and the refinement was continued for a few cycles of block-diagonal least squares (BDLS) in which the matrix was divided into five blocks. A difference map calculated at this point clearly showed all the hydrogen atoms. Several additional cycles of BDLS which included the hydrogen atoms (isotropic temperature factors) were run. The refinement was stopped when the shifts in the parameters of the heavier atoms were all less than one-sixth of their respective standard deviations (one-third of a standard deviation for the hydrogens). A difference Fourier based on the final parameters showed no features greater than 0.25 e Å^{-3} in magnitude except for four holes ranging

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from −0.3 to 0.4 e Å^{-3} about the bromide ion. The final conventional R factor is 2.4%.[21]

The structure of **11**·HBr was solved and refined in an analogous manner. Its final difference Fourier has no features greater than 0.2 e Å^{-3} in magnitude. The final R factor is 2.3%.[21]

The absolute configurations of **3**·HBr and **11**·HBr were established by comparison of the observed and calculated structure factors of the significant Bijvoet pairs of reflections[22] for each salt. For **3**·HBr there were 24 pairs of reflections for which $F_o(hkl)$ and $F_o(\bar{hkl})$ were both greater than 30 e and $|\Delta F_o| = ||F_o(hkl)| - |F_o(\bar{hkl})||$ was greater than 1.6 e. The corresponding differences in the calculated structure factors, ΔF_c, agreed in sign with their respective ΔF_o for all 24 pairs. For **11**·HBr, the signs of all but one of the ΔF_c agreed with the signs of the corresponding ΔF_o for the 48 pairs of reflections with F_o greater than 15 e and ΔF_o greater than 1.9 e.

(21) Listings of structure factors, coordinates, and thermal parameters for **3**·HBr and **11**·HBr will appear following these pages in the microfilm edition of this volume of the Journal. Single copies may be obtained from the Reprint Department, ACS Publications, 1155 Sixteenth St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for micofiche.

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Thiophosphate Analogs of Nucleoside Di- and Triphosphates

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Abstract: The chemical synthesis of thiophosphate analogs of nucleoside di- and triphosphates bearing a sulfur at the terminal phosphorus atom by the use of *S*-2-carbamoylethyl thiophosphate is described. They can readily be oxidized to their disulfides and are only slowly degraded by alkaline phosphatase. Their reactivity with 5,5′-dithiobis(2-nitrobenzoic acid) was investigated and compared with that of other nucleoside thiophosphates.

Nucleotide analogs modified by replacement of an oxygen of the phosphate group by sulfur have recently been described, and have shown interesting behavior with enzymes involved with nucleic acid metabolism.[1] Analogs of nucleotide anhydrides bearing a sulfur at the α phosphorus atom have also been prepared, and the triphosphates have been used as substrates for DNA-dependent RNA polymerase to obtain polynucleotides containing a thiophosphate backbone.[2] Adenosine 5′-*O*-(1-thiotriphosphate) is also a substrate for C-C-A pyrophosphorylase, and using this enzyme adenosine 5′-*O*-phosphorothioate can be incorporated into $tRNA^{Phe}$.[3] The compound is, however, a competitive inhibitor for phenylalanyl-tRNA synthetase. These examples illustrate the usefulness of the 1-thiophosphate analogs, but a difficulty arises in their use in kinetic studies with enzymes, since the compounds exist as diastereoisomeric pairs due to the asymmetry at the α phosphorus atom. It is possible for the two isomers to have quite different behavior toward a particular enzyme, and this can present difficulties in interpretation of kinetic results. In the extreme, it is possible that one diastereoisomer is a substrate, while the other is an inhibitor. An analogy for this is provided by the *O*-(*p*-nitrophenyl) ester of thymidine 5′-*O*-thiophosphate, one diastereoisomer of which is a substrate for snake venom phosphodiesterase, while the other is an inhibitor.[4] When the isomers can be separated, as is the case with uridine 2′,3′-*O*,*O*-cyclophosphorothioate,[5] the isomerism can be useful to help define the stereochemistry of enzymatic processes.[6] However, for di- and triphosphates, which are difficult to crystallize, separation of the isomers is likely to be impossible. It was therefore thought of interest to attempt the preparation of di- and triphosphate analogs bearing sulfur on the terminal phosphorus atom, since these compounds would have no asymmetry at this site. The compounds were also of interest as potential affinity labels for enzymes containing thiol or disulfide groups in the region of their active site, because of the possibility of disulfide formation with the thiophosphate residue.

Preparation of nucleoside 5′-di- and -triphosphates is normally achieved by activation of a nucleoside 5′-phosphate followed by attack on the activated product using either orthophosphate or pyrophosphate ion. Because of the possibility of attack of thiophosphate ion on such a system *via* either sulfur or oxygen, two possible products can be envisaged from this type of reaction, one having the sulfur on the terminal phosphorus, and the other having a sulfur bridge between the two phosphorus atoms. The first activation method employed was that using *N*,*N*′-carbonyldiimidazole.[7] The reaction of adenosine 5′-phosphoroimidazolate with thiophosphate ion under the reported conditions gave no diphosphate-like product, even after allowing the reaction mixture to stand for a much longer time than is

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