[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, AND NEW YORK STATE PSYCHIATRIC INSTITUTE]

Synthesis of Several Oxazolidines and Cycloacetals of Sphingosine and Dihydrosphingosine.[1] IV

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Several substituted oxazolidines of sphingosine and dihydrosphingosine were prepared by condensation with benzaldehyde to form the *N*-benzylidene derivative, after which ring closure was effected by reaction with the appropriate acyl chloride in aqueous alkali and ether. The formation of the oxazolidine ring involved the hydroxyl group on carbon atom 3 of sphingosine or dihydrosphingosine without change in configuration. An oxazolidine bearing no substituent in the 3-position was prepared by catalytic reduction of *erythro*-2-phenyl-3-carbobenzoxy-4-hydroxymethyl-5-(1-*trans*-pentadecenyl)oxazolidine to yield the corresponding *erythro*-2-phenyl-4-hydroxymethyl-5-pentadecyloxazolidine. Reaction of *erythro*-2-phenyl-3-carbobenzoxy-4-hydroxymethyl-5-(1-*trans*-pentadecenyl)oxazolidine with osmium tetroxide yielded a dihydro, instead of the anticipated dihydroxy, derivative. The oxazolidine ring, along with its tertiary amide group, was stable to reduction by lithium aluminum hydride. A few *N*-substituted sphingosines were allowed to react with benzaldehyde to yield the corresponding *N*-acylsphingosine-1,3-cycloacetals; these remained intact during hydrogenation to give the respective dihydro compounds.

The synthesis of an oxazolidine of sphingosine or dihydrosphingosine was undertaken in an effort to obtain an appropriate intermediate in which the primary hydroxyl group would be free for the preparation of various 1-substituted derivatives of these long chain bases.[2] This was accomplished by reacting the free base with benzaldehyde to form the *N*-benzylidene compound, the structure of which was determined both by the absence of a positive ninhydrin reaction and by an absorption maximum at 247 mμ[3] (Table I). Destruction of the azomethine linkage by catalytic hydrogenation of *N*-benzylidene sphingosine yielded the expected *N*-benzyldihydrosphingosine which still gave a negative ninhydrin reaction but exhibited no absorption at 247 mμ. *p*-Nitrobenzylidene sphingosine (Table I) showed an absorption shift to the red with a maximum at 285 mμ presumably as a result of the increased conjugation of the system owing to the presence of the nitro group.[4]

Cyclization of the *N*-benzylidene derivative was effected in aqueous alkali and ether by treatment with the appropriate acyl chloride to yield the corresponding substituted oxazolidine (I). Only fair yields were obtained in this step (Table I); an examination of a few parameters of this reaction showed that 1) insufficient cooling and inadequate mixing of the two phases of the reaction mixture, 2) slow addition of the acyl chloride, or 3) incomplete drying of the product remaining after ether removal resulted in lower yields. This was attributed to cleavage of the sensitive azomethine linkage, as the amide of the base was isolated in several instances. Indeed, contaminating amide was always a competing side product of the reaction. Although no effort was directed at separating the two diastereomers expected to be formed, it was assumed that both were likely to be present in the final product. All of the oxazolidines prepared exhibited little or no absorption either at 247 mμ or 285 mμ but showed the characteristic infrared bands between 1100–1200 cm.[1] (Table I).[5] In order to determine the locus of the acyl substituent and the occurrence of any rearrangements, *erythro*-2-phenyl-3-carbobenzoxy-4-hydroxymethyl-5-(1-*trans*-pentadecenyl)oxazolidine, the corresponding 3-acetyl oxazolidine, and *erythro*-2-phenyl-3-benzoyl-4-hydroxymethyl-5-pentadecyloxazolidine were treated at room temperature with dilute acid. *N*-Carbobenzoxysphingosine, *N*-acetyldihydrosphingosine (the *N*-acetylsphingosine initially obtained was identified after hydrogenation), and *N*-benzoyldihydrosphingosine, respectively, were isolated from the reaction mixtures. This result confirmed the amide nature of the acyl linkage and precluded the occurrence of an allylic rearrangement or inversion at carbon atom 3 (it was not known, yet, whether or not the hydroxyl group at this position was involved in ring closure during oxazolidine formation).

Elucidation of the oxazolidine structure was

(1) This investigation was supported in part by research grant No. B-344 (C5 and C6) from the Institute of Neurological Diseases and Blindness of the National Institutes of Health, Public Health Service.

(2) While this work was in progress, D. Shapiro, H. M. Flowers, and S. Spector-Shefer, *J. Am. Chem. Soc.*, 81, 3743 (1959) reported the total synthesis of dihydrosphingomyelin. Their procedure utilized as a key intermediate *cis*-2-phenyl-4-hydroxymethyl-5-pentadecyl-2-oxazoline which was prepared by the cyclization of methyl *threo*-α-benzamido-β-hydroxystearate with thionyl chloride followed by reduction with lithium aluminum hydride. The unsaturated sphingomyelins were prepared by the same investigators, *J. Am. Chem. Soc.*, 81, 4360 (1959), employing as intermediate, *cis*-2-phenyl-4-hydroxymethyl-5-(1-pentadecenyl)-2-oxazoline.

(3) G. E. McCasland and E. C. Horswill, *J. Am. Chem. Soc.*, 73, 3923 (1951).

(4) E. A. Braude, E. R. H. Jones, and G. G. Rose, *J. Chem. Soc.*, 1104 (1947).

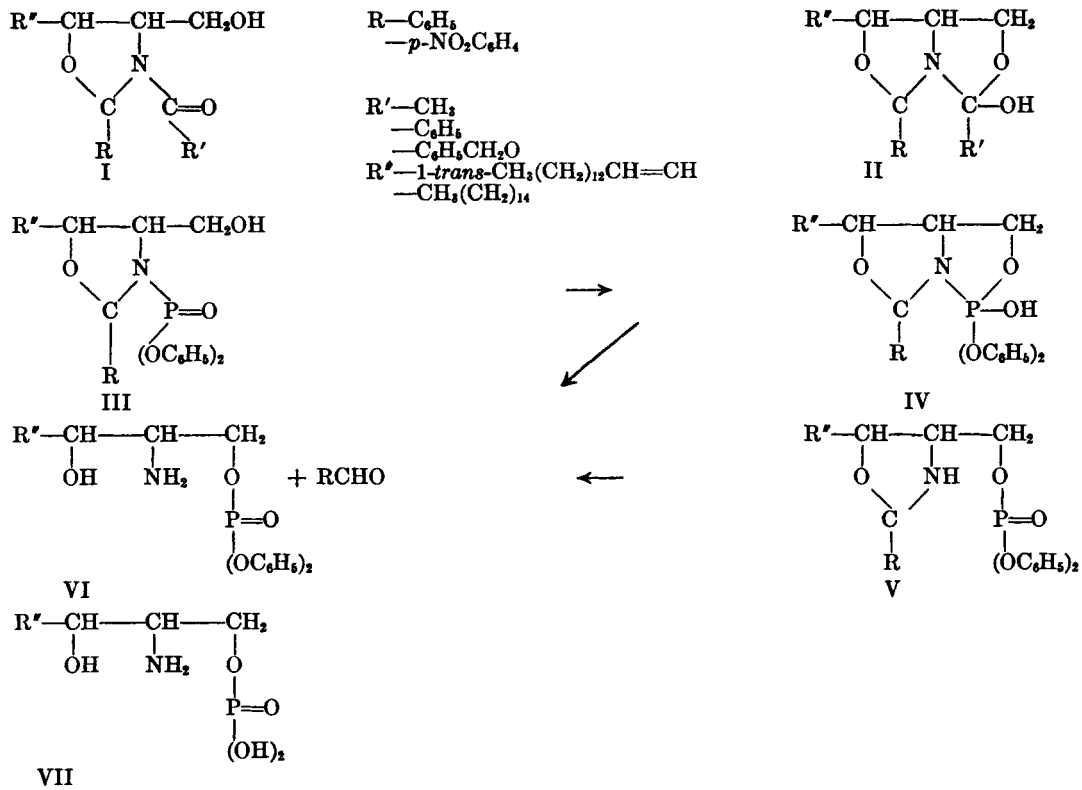
(5) E. D. Bergmann and H. Resnick, *J. Chem. Soc.*, 1662 (1956).

TABLE I

Compound	Empirical Formula	Mol. Wt.	Yield, %	M.P.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
1. *N*-Benzylidene sphingosine[a]	$C_{25}H_{41}O_2N$	387.4	65	52–55	77.44	76.98	10.66	10.64	3.61	3.55
2. *N*-*p*-Nitrobenzylidene sphingosine[a1]	$C_{25}H_{40}O_4N_2$	432.3	63	69–72	69.39	69.47	9.33	9.28	6.48	6.63
3. *N*-Benzylidene dihydrosphingosine[a2]	$C_{25}H_{43}O_2N$	389.3	77	46–49	77.05	76.79	11.13	10.91	3.60	3.57
4. *N*-Benzyldihydrosphingosine	$C_{25}H_{45}O_2N$	391.4	80	63–65	76.65	76.38	11.59	11.61	3.58	3.49
5. *Erythro*-2-phenyl-3-acyl-4-hydroxymethyl-5-(1-*trans*-pentadecenyl)oxazolidine										
a. 3-Acetyl[b]	$C_{27}H_{43}O_3N$	429.3	32	128–130	75.46	75.52	10.10	10.17	3.26	3.16
b. 3-Benzoyl[c,d]	$C_{32}H_{45}O_3N$	491.4	64	133–136	78.15	77.80	9.23	9.48	2.85	2.84
c. 3-Carbobenzoxy[c]	$C_{33}H_{47}O_4N$	521.4	55	99–101	75.95	75.93	9.09	8.96	2.69	2.70
6. *Erythro*-2-*p*-nitrophenyl-3-acyl-4-hydroxymethyl-5-(1-*trans*-pentadecenyl) oxazolidine										
a. 3-Benzoyl[d1]	$C_{32}H_{44}O_5N_2$	536.4	44	202–203	71.59	71.27	8.27	8.23	5.22	5.09
b. 3-Carbobenzoxy	$C_{33}H_{46}O_6N_2$	566.4	35	103–105	69.91	70.22	8.19	8.30	4.94	4.89
7. *Erythro*-2-phenyl-3-acyl-4-hydroxymethyl-5-pentadecyloxazolidine										
a. 3-Acetyl[b,c,e]	$C_{27}H_{45}O_3N$	431.4	32	149–151	75.11	74.86	10.52	10.46	3.25	3.20
b. 3-Benzoyl[b,c,e]	$C_{32}H_{47}O_3N$	493.4	57	145–147	77.83	77.96	9.60	9.75	2.84	2.83
c. 3-Carbobenzoxy[e]	$C_{33}H_{49}O_4N$	523.4	60	122–123	75.66	75.48	9.44	9.63	2.67	2.70
d. 3-Diphenylphosphoryl[f]	$C_{37}H_{52}O_5NP$	621.5	74	122–123	71.44	71.60	8.43	8.52	2.25	2.21
8. *Erythro*-2-phenyl-4-hydroxymethyl-5-pentadecyloxazolidine[b]	$C_{25}H_{43}O_2N$	389.3	89	58–60	77.05	76.94	11.13	11.27	3.60	3.62
9. *N*-Acylsphingosine-1,3-cycloacetal of benzaldehyde										
a. *N*-Acetyl	$C_{27}H_{43}O_3N$	429.3	26	123–125	75.46	75.92	10.10	10.01	3.26	3.18
b. *N*-Benzoyl[b]	$C_{32}H_{45}O_3N$	491.4	51	135–138	78.15	77.92	9.23	9.36	2.85	2.84
c. *N*-Carbobenzoxy	$C_{33}H_{47}O_4N$	521.4	50	103–106	75.95	75.64	9.09	9.11	2.69	2.66
10. *N*-Carbobenzoxysphingosine-1,3-cycloacetal of *p*-nitrobenzaldehyde	$C_{33}H_{46}O_6N_2$	566.4	41	108–111	69.91	69.79	8.19	8.26		
11. *N*-Acyldihydrosphingosine 1,3-cycloacetal of benzaldehyde										
a. *N*-Benzoyl	$C_{32}H_{47}O_3N$	493.4	70	150–151	77.83	77.98	9.60	9.68	2.84	2.83
b. *N*-Carbobenzoxy	$C_{33}H_{49}O_4N$	523.4	80	123–125	75.66	75.79	9.44	9.64	2.67	2.61

[a, a1, a2] Molar extinction coefficients at 247 mμ are 12,900, 13,200 (285 mμ), and 12,700, respectively. [b] Infrared absorption bands are as follows: 5a-1195(w), 1185(w), 1145(m), 1120(s), 1100(w) cm.$^{-1}$; 7a-1195(m), 1185(m), 1160(m), 1145(m), 1120(s), 1100(m) cm.$^{-1}$; 7b-1195(m), 1185(m), 1160(s), 1140(s), 1125(s), 1100(w) cm.$^{-1}$; 8-1195(m), 1175(w), 1150(w), 1140(m), 1120(w) cm.$^{-1}$; 9b-1165(s), 1135(w), 1125(s) cm.$^{-1}$ [c] The percentage of the theoretical amount of active hydrogen found for compounds 5b, 5c, 7a and 7b was 110, 112, 96, and 105, respectively. [d,d1] crystallized from ethanol and ethanol-ethyl acetate, (1:1) respectively. [e] $(\alpha)_D^{22}$ for 7a, 7b, and 7c are + 29.2, + 38.3, and + 30.8 (*c*, 2 in chloroform), respectively. [f] Calculated; P, 4.98; found: P, 5.03. [g] Crystallized from *n*-heptane-ethanol (9:1).

begun by hydrogenating several *N*-substituted oxazolidines of sphingosine and comparing them with the corresponding compounds derived directly from the saturated base. In view of the different reactivities of the hydroxyl groups in sphingosine and dihydrosphingosine, it was anticipated that ring closure would favor the allylic hydroxyl group in the former compound and the primary hydroxyl group in the latter with a resulting dissimilarity in physical properties. However, the hydrogenated forms of 5a, 5b, and 5c could not be distinguished on the basis of melting point or optical rotation from compounds 7a, 7b, and 7c, respectively, (Table I). In addition, the infrared spectra of 5a and 5b were identical with 7a and 7b, respectively, and it could not be determined unequivocally, because of overlapping of bands, whether or not the primary or secondary hydroxyl group was free. The NMR spectrum of 5c was indistinguishable from that of 7c.[20] An attempt was made to methylate the free hydroxyl group of *erythro*-2-phenyl-3-carbobenzoxy-4-hydroxymethyl-5-(1-*trans*-pentadecenyl)oxazolidine with the intention of subjecting the product successively to acid hydrolysis, hydrogenation, and periodic acid oxidation. However, all attempts to methylate this compound, as well as several analogous members of the series, were unsuccessful



even under the permethylation conditions of Bredereck.[6] Neither could this group be acetylated, benzoylated, diphenylphosphorylated, condensed with α-bromo-2,3,4,6-tetraacetylglucose, or oxidized by aluminum isopropoxide in either benzene-acetone or toluene-cyclohexanone. In all instances, the initial compound was recovered unaltered from the reaction medium. Active hydrogen determinations with lithium aluminum hydride[7] on several compounds (Table I) yielded values ranging from 96 to 112%. The refractory quality of this hydroxyl group suggested that it was not free but, perhaps, involved in a hemiacetal structure with the adjacent carbonyl group of the tertiary amide (II). This would account for its apparent unreactivity toward electrophilic reagents, as this linkage is alkali stable. It was observed that in a related type of compound in which no carbonyl group was even present,[2] 2-phenyl-4-hydroxymethyl-5-pentadecyl-2-oxazoline, the hydroxyl group had low reactivity, failing to react with dibenzylchlorophosphate but reacting readily with stronger phosphorylating agents.

If the hemiacetal structure is present, it might be utilized in the determination of the oxazolidine structure by replacement of the acyl group at position 3 with another function having greater polarizability[8] such as an organophosphorus group. In order to accomplish this, it proved necessary to prepare a free oxazolidine—*i.e.*, with no substituent other than hydrogen on the nitrogen atom. Although the hypothesis has been advanced[2] that the existence of a simple oxazolidine of well established structure and purity is doubtful and that the presence of an alkyl or acyl group in the 3-position is necessary to prevent rearrangement to the Schiff base,[9] several simple crystalline oxazolidines have been prepared recently from compounds in the chloramphenicol series by Bergmann and Resnick.[5] When *erythro*-2-phenyl-3-carbobenzoxy-4-hydroxymethyl-5-(1-*trans*-pentadecenyl)oxazolidine or the corresponding saturated compound was hydrogenated over platinum in glacial acetic acid (in ethanol–ethyl acetate (1:3) only reduction of the double bond occurred), there was obtained in each instance a compound melting at 58–60°, exhibiting no absorption in the ultraviolet at 247 mμ, showing the characteristic oxazolidine infrared bands (Table I) and having no tertiary amide band at 1640 cm.$^{-1}$. Acetylation of either of these compounds with acetic anhydride in pyridine yielded the *N*-acetyl oxazolidine whose melting point was the same as that of the product obtained from the reaction of acetyl chloride with *N*-

(6) H. Bredereck, *Ber.*, **87**, 35 (1954).

(7) These analyses, as well as those for carbon and hydrogen, were performed by the Schwarzkopf Microanalytical Laboratory.

(8) H. Tolkmith, *Ann. N.Y. Acad. Sci.*, **79**, 187 (1959).

(9) See the following references for a review of this subject: E. D. Bergmann, *Chem. Rev.*, **53**, 309 (1953). J. W. Cornforth in R. C. Elderfield, *Heterocyclic Compounds*, **5**, 393 (1957).

benzylidene dihydrosphingosine; *N*-benzylidene sphingosine in pyridine does not undergo cyclization to the oxazolidine in the presence of acetic anhydride. Reaction with dimethyl sulfate in aqueous alkali provided mainly the *N*-methyl compound with traces of *O*-methyl. The same simple oxazolidines, derived either from sphingosine or dihydrosphingosine, on reaction with diphenylphosphoryl chloride in the usual manner gave crystalline compounds of similar melting points and containing one atom of phosphorus (III). Treatment with dilute acid followed by hydrogenolysis of the protective groups yielded in both instances dihydrosphingosine-1-phosphate, the position of the phosphate group being established by procedures employed previously.[10] Thus, ring closure in oxazolidine formation from *N*-benzylidene sphingosine and *N*-benzylidene dihydrosphingosine involved the same hydroxyl group, the one on carbon atom 3. It was shown from the infrared band in the primary hydroxyl group region that the secondary hydroxyl group of 1-*p*-nitrophenylpropane-2-amino-1,3-diol participated in oxazolidine formation.[5]

Intramolecular rearrangements of organophosphorus compounds are well known[8,11] and the mechanism for the rearrangement described above may be most easily explained by assuming the formation of a cyclic triester phosphate intermediate (IV) analogous to the hemiacetal structure previously formulated (II). In the presence of acid, the high energy nitrogen-phosphorus bond would be rapidly cleaved to yield the more stable isomer (VI). The product isolated at this stage was semisolid and could not be adequately characterized; phosphorus values were approximately 90% and no positive ninhydrin reaction was obtained, possibly because the amino group may have been present in salt form resulting from the hydrolysis. Hydrogenolysis of the protective groups provided dihydrosphingosine-1-phosphate (VII) presumably, containing some dihydrosphingosine as contaminant which was removed by alkaline purification. It has been assumed in this mechanism that the phosphate group is initially (or, possibly, immediately after the addition of acid) stabilized by ring ester formation with the free primary hydroxyl group and that opening of the oxazolidine ring occurred either simulatneously with (VI) or subsequently to (V) the splitting of the phosphoramide bond. If the phosphate group were not previously stabilized as suggested, then, most likely, it would have been lost from the molecule under the conditions of temperature and acidity employed (heating to boiling with 0.36*N* sulfuric acid in 95% ethanol), as Zervas and Katsoyannis[12] found that phosphate is released almost quantitatively from *N*-phosphoroamino acids within five to ten minutes at *p*H 4.0 and at 25–30°. Also, if the oxazolidine ring were opened before cyclic ester formation of the phosphate group, it would be necessary to assume that the nitrogen-phosphorus bond remained intact under these conditions and that a mixture of phosphate esters would occur since cyclization through either hydroxyl group of the base would be possible. No evidence of the 3-isomer was obtained by periodic acid oxidation.[10] The entire question of the sequence of events regarding ring opening and phosphate migration might have been more readily answered had the initial oxazolidine been hydrogenated first and then subjected to ring scission. However, when this was tried, the product obtained did not behave like a dihydrosphingosine phosphate since it was completely soluble in acetone. The presence of the phenyl ester phosphate bonds favors migration by resonance stabilization of the cyclic phosphate ester linkage formed from the primary hydroxyl group and the coordinate covalent P→O bond.

Prior to the preparation of the free oxazolidine, as an alternative procedure for structure determination, an attempt was made to prepare an oxazolidine of sphingosine in which the direction of ring closure would be unequivocal and to compare its infrared spectrum with those obtained previously. For this purpose, 3-*O*-methylsphingosine II was chosen; it gave the *N*-benzylidene derivative in good yield but in the subsequent reaction with benzoyl chloride, cyclization could not be effected, and the product isolated was 3-*O*-methyl-*N*-benzoylsphingosine II, which corresponded with the compound obtained by the direct benzoylation of 3-*O*-methylsphingosine II.

The determination of structure was approached also by attempting to hydroxylate the double bond of *erythro*-2-phenyl-3-carbobenzoxy-4-hydroxymethyl-5-(1-*trans*-pentadecenyl)oxazolidine. In the presence of osmium tetroxide and catalytic amounts of pyridine,[13] the anticipated dark brown adduct was obtained which, when cleaved with aqueous alcoholic sodium sulfite in the usual manner, yielded a compound melting at 122–123° and resisting oxidation with neutral periodate. Acid hydrolysis of this compound resulted in a product which proved to be *N*-carbobenzoxydihydrosphingosine. Therefore, the oxazolidine obtained after reaction with osmium tetroxide must have been the dihydro derivative. As the hydrolysis of the untreated oxazolidine yielded *N*-carbobenzoxysphingosine, m.p. 78–79° (see Experimental), the *N*-carbobenzoxydihydrosphingosine obtained from the reaction product must have been formed during the osmylation. No other compound could be isolated from the reaction mixture. This repre-

(10) B. Weiss, *J. Am. Chem. Soc.*, 79, 5553 (1957).

(11) F. L. Pizer and C. E. Ballou, *J. Am. Chem. Soc.*, 81, 915 (1959).

(12) L. Zervas and P. G. Katsoyannis, *J. Am. Chem. Soc.*, 77, 5351 (1955).

(13) R. Criegee, B. Marchand, and H. Wannowius, *Ann.*, 550, 99 (1942).

sents a novel reaction of osmium tetroxide in which the total reduction of a double bond is effected. It is evident that after the formation of the cyclic osmic ester intermediate[14] cleavage of the carbon-oxygen bond rather than the oxygen-osmium bond occurs. It would be of interest to determine by the use of deuterium or tritium oxide whether or not the reduction is accompanied by a retention of configuration.

Hydrogenation of a sphingosine derivative bearing a substituent on the allylic hydroxyl group results in a mixture of reduction products owing to the hydrogenolysis of the allylic grouping.[15] Incorporation of the allylic function into the oxazolidine ring resulted in a marked stabilizing effect, as no cleavage products were observed following hydrogenation. This stability was shown also by several *N*-substituted 1,3-cycloacetals of sphingosine, which were prepared for purposes of comparison with the oxazolidines. Neither cleavage of the oxazolidine ring of dihydrosphingosine nor reduction of the tertiary amide group was effected by refluxing with lithium aluminum hydride in diethyl ether. Because scission of this heterocyclic ring has been described,[16] the difference in results may be due to greater ring stability of the present compounds or to the lower operating temperature, as dioxane was employed as solvent in the earlier work. The acetal linkage also was unaffected by lithium aluminum hydride, as has been described previously.[17] However, *N*-benzoyldihydrosphingosine was readily reduced by this reagent to *N*-benzyldihydrosphingosine, which was identical with the product obtained by the hydrogenation of *N*-benzylidene sphingosine.

Erythro-2-phenyl-3-benzoyl-4-hydroxymethyl-5-(1-*trans*-pentadecenyl)oxazolidine exhibited no carcinostatic effect toward a variety of transplantable mouse tumors. When incorporated into the feeding fluids of tissue cultures of human glioblastoma multiforme at final concentrations of $10^{-3}M$, neither the above compound nor the *N*-benzoylsphingosine-1,3-cycloacetal of benzaldehyde caused any damage to the tumor cells after 7 days exposure.[18]

EXPERIMENTAL

N-Benzylidene-and p-nitrobenzylidene sphingosine and N-benzylidene dihydrosphingosine (I). Sphingosine sulfate, 7.0 g., was converted to the free base as previously described[10] and dried thoroughly over phosphorus pentoxide. The base was dissolved in 350 ml. of benzene, C.P., containing either 2.0 ml. of freshly distilled benzaldehyde or 3.1 g. of *p*-nitrobenzaldehyde, and the reaction mixture was azeotropically distilled while connected to a water separator,[19] fresh benzene being added as needed. When the separation of water ceased, the solution was concentrated to a sirup under diminished pressure, 50 ml. of *n*-heptane were added, and the solution was reconcentrated to remove the last traces of benzene. The sirup was taken up in 40 ml. of *n*-heptane and chilled overnight at 5°. The product was dried over paraffin and employed directly in the next synthetic step or recrystallized from the same solvent (and volume) for ultraviolet absorption determinations.[20] *N*-Benzylidene dihydrosphingosine was prepared in the same manner after reduction of sphingosine to the dihydro form[10] (Table I). Each compound gave a negative ninhydrin reaction in methanol.

N-Benzyldihydrosphingosine (II). *N*-Benzylidene sphingosine (1), 3.1 g., was reduced over 150 mg. of platinum oxide in 75 ml. of ethanol–ethyl acetate (3:1). After reduction, the reaction mixture was warmed and filtered with suction. The catalyst was washed on the filter with 50 ml. of warm ethanol, and the combined filtrate and wash was concentrated under diminished pressure. The residue, after being reconcentrated from 50 ml. of *n*-heptane, was crystallized from 100 ml. of the latter solvent (Table I). The product exhibited negligible absorption at 247 mμ and was ninhydrin negative. The same compound was obtained by the reduction of *N*-benzoyldihydrosphingosine with lithium aluminum hydride.[21]

Erythro-2-phenyl (or p-nitrophenyl)-3-acyl-4-hydroxymethyl-5-(1-trans-pentadecenyl)oxazolidine (III). *N*-Benzylidene sphingosine, 3.1 g., or 3.5 g. of *p*-nitrobenzylidene sphingosine (I) (8.0 mmoles) was dissolved in 300 ml. of ether and chilled in an ice bath. After the addition of 60 ml. of 0.25*N* sodium hydroxide, the solution was vigorously stirred while 11.0 mmoles of acyl chloride in 35 ml. of ether were added promptly over a 3-min. period. The ether phase was removed after 5 min. of additional stirring, washed with 100 ml. of water, and concentrated under reduced pressure. The residue was dried thoroughly over phosphorus pentoxide *in vacuo* and crystallized from *n*-heptane and then from 83% ethanol (1.0 g. per 100 ml. solvent) (Table I).

The reaction of 3-*O*-methyl-*N*-benzylidene sphingosine II (extinction coefficient 16,300 at 247 mμ) with benzoyl chloride yielded 3-*O*-methyl-*N*-benzoylsphingosine II, m.p., 95–96°.

Anal. Calcd. for $C_{26}H_{43}O_3N$ (417.3): C, 74.76; H, 10.38; N, 3.35. Found: C, 74.12; H, 10.37; N, 3.38.

Erythro-2-phenyl-3-acyl-4-hydroxymethyl-5-pentadecyloxazolidine (IV). This series of compounds was prepared either by treatment of 3.1 g. of *N*-benzylidene dihydrosphingosine (I) with the appropriate acyl chloride as described in III (Table I) or by hydrogenation of 1.0 g. of the corresponding unsaturated product from III in 100 ml. of ethanol–ethyl acetate (1:1) containing 150 mg. of platinum oxide in the

(14) W. A. Waters in H. Gilman, *Organic Chemistry, An Advanced Treatise*, Vol. IV, 1120, John Wiley and Sons, Inc., New York, 1953.

(15) H. E. Carter, O. Nalbandov, and P. A. Tavormina, *J. Biol. Chem.*, **192**, 197 (1951).

(16) E. D. Bergmann, D. Lavie, and S. Pinchas, *J. Amer. Chem. Soc.*, **73**, 5662 (1951).

(17) C. S. Marvel and H. W. Hill, Jr., *J. Am. Chem. Soc.*, **73**, 481 (1951).

(18) These studies were made through the efforts of Dr. Erich Hirschberg, Departments of Biochemistry, Medicine, and Surgery and Institute of Cancer Research, Columbia University College of Physicians and Surgeons, New York, N. Y.

(19) M. Renoll and M. S. Newman, *Org. Syntheses*, **Coll. Vol. III**, 502 (1955).

(20) The ultraviolet determinations were made with a Beckman DU spectrophotometer; samples were diluted to approximately 2.5×10^{-5} moles per liter with either ethanol or ethanol:chloroform (200:1) and read against the appropriate solvent blank. The infrared spectra were obtained on potassium bromide disks with a Perkin Elmer Model 21 Double Beam Infrared Spectrophotometer through the courtesy of Drs. S. Lieberman and S. Solomon, Department of Biochemistry, Columbia University. The NMR spectra were made possible through the generous offer of Mr. L. F. Johnson, Varian Associates, Palo Alto, Calif.

(21) B. Weiss, *J. Am. Chem. Soc.*, **80**, 4657 (1958).

manner mentioned in II. The crude product was crystallized from ethanol (1.0 g. per 75 ml. solvent).

Osmylation. Erythro-2-phenyl-3-carbobenzoxy-4-hydroxymethyl-5-(1-*trans*-pentadecenyl)oxazolidine, 1.90 g., in 60 ml. of anhydrous ether containing 2 ml. of pyridine and 1.0 g. of osmium tetroxide was allowed to stand 3 days at room temperature. The reaction mixture was concentrated to a sirup under reduced pressure and then refluxed 6 hr. after the addition of 150 ml. of ethanol and 5.0 g. of sodium sulfite in 30 ml. of water. Upon the addition of 300 ml. of ethyl acetate, the solution was successively filtered, concentrated to a paste, redissolved in 500 ml. of ether–ethyl acetate (1:1), and then washed with three 100-ml. portions of water. The residue, obtained upon reconcentration, was crystallized from 310 ml. of 75% ethanol. When necessary, the product was freed of colored contaminant by treatment with Amerlite IRA-400 in ethanol followed by filtration and precipitation of the product from the filtrate by the addition of the required amount of water; yield of *erythro*-2-phenyl-3-carbobenzoxy-4-hydroxymethyl-5-pentadecyloxazolidine, 0.70 g. (31%) m.p. 122–123° (Table I).

Anal. Calcd. for $C_{33}H_{49}O_4N$ (523.4): C, 75.66; H, 9.44; N, 2.67; active hydrogen, 0.19. Found: C, 75.48; H, 9.52; N, 2.65; active hydrogen, 0.20.

Acid hydrolysis according to V of the above compound gave *N*-carbobenzoxydihydrosphingosine, m.p., 106–107°.

Anal. Calcd. for $C_{26}H_{45}O_4N$ (435.6): C, 71.62; H, 10.41; active hydrogen, 0.69. Found: C, 71.23; H, 10.46; (active hydrogen, 0.66).

Hydrolysis of oxazolidine (V). Oxazolidine, 1 g., in 99 ml. of 95% ethanol containing 1.0 ml. of concd. sulfuric acid, was heated for 3 min. in a boiling water bath and allowed to stand 4 hr. at room temperature. After the addition of 125 ml. of water to the clear reaction mixture, the flocculent precipitate was removed by suction filtration, dried over phosphorus pentoxide, and crystallized from the appropriate solvent.[10] The filtrate remaining after removal of the initial precipitate was treated in the usual manner with 2,4-dinitrophenylhydrazine; the product was crystallized from 10 ml. of glacial acetic acid. Yield of *N*-acetylsphingosine (identified as *N*-acetyldihydrosphingosine) after hydrolysis of *erythro*-2-phenyl-3-acetyl-4-hydroxymethyl-5-(1-*trans*-pentadecenyl)oxazolidine was 0.14 g., m.p. 119°; yield of *N*-carbobenzoxysphingosine (crystallized from petroleum ether, b.p. 60–70°) from the corresponding 3-carbobenzoxyoxazolidine was 0.33 g., m.p. 78–79°; yield of *N*-benzoyldihydrosphingosine from *erythro*-2-phenyl-3-benzoyl-4-hydroxymethyl-5-pentadecyloxazolidine was 0.48 g., m.p. 119–121°.

Anal. Calcd. for $C_{25}H_{43}O_3N$ (405.3): C, 74.10; H, 10.69; N, 3.45. Found: C, 73.63; H, 10.49; N, 3.45.

The 2,4-dinitrophenylhydrazone isolated melted at 242–244° in agreement with the derivative formed from authentic benzaldehyde.

Erythro-2-phenyl-4-hydroxymethyl-5-pentadecyloxazolidine (VI). *Erythro*-2-phenyl-3-carbobenzoxy-4-hydroxymethyl-5-pentadecyloxazolidine, 1.28 g., was hydrogenated over 150 mg. of platinum oxide in 50 ml. of glacial acetic acid. After the uptake of hydrogen ceased, the reaction mixture was filtered with gravity, and the catalyst washed with 10 ml. of warm glacial acetic acid. To the combined filtrate and wash, surrounded by an ice bath, were added 300 ml. crushed ice water followed by 25% sodium hydroxide until the solution became alkaline. The precipitate, upon aggregation after several minutes standing, was removed by suction filtration, washed with 400 ml. of ice cold water, and dried over phosphorus pentoxide. The melting point remained unchanged after crystallization from ethanol-water (3:1) (100 mg. per 4 ml. solvent) (Table I). No absorption was shown in the ultraviolet at 247 mμ and a negative ninhydrin reaction, obtained in methanol, became positive after the addition of water and prolonged heating.

To 200 mg. of the free oxazolidine (VI) in 50 ml. of pyridine was added 0.5 ml. of acetic anhydride. After standing 30 min. at room temperature, the reaction mixture was diluted with 25 ml. of water, chilled, and filtered, and the precipitate crystallized from ethanol; yield of *erythro*-2-phenyl-3-acetyl-4-hydroxymethyl-5-pentadecyloxazolidine, 190 mg.; m.p. 149–151°.

Similarly, the 3-diphenylphosphoryl substituted oxazolidine was prepared by the addition of 3.56 g. of diphenylphosphoryl chloride[22] (1.32 mmoles) to 2.60 g. of the free oxazolidine (0.66 mmole) in 30 ml. of anhydrous pyridine, previously chilled in an ice bath. After standing overnight at 5°, the yellow orange solution, which had deposited a heavy precipitate of pyridine hydrochloride, was poured into 200 ml. of ice water. The precipitate was filtered with suction, washed on the filter with 500 ml. of cold water, and crystallized twice from 100 ml. of 85% ethanol (Table I).

N-Acylsphingosine-1,3-cycloacetal of benzaldehyde and p-nitrobenzaldehyde (VII). The appropriate *N*-acylsphingosine,[10] 10.0 mmoles, was dissolved in 350 ml. of benzene containing 20.0 mmoles of benzaldehyde or *p*-nitrobenzaldehyde and 0.15 ml. of concd. sulfuric acid, and the reaction mixture was azeotropically distilled as described in I. After concentration of the solution as in I, the residue was crystallized twice from *n*-heptane (Table I).

N-Acyldihydrosphingosine-1,3-cycloacetal of benzaldehyde (VIII). Compound VII, 1.0 g., was hydrogenated according to the procedure described in II; the residue was crystallized from *n*-heptane (Table I). The carbobenzoxy group remained intact after hydrogenation of *N*-carbobenzoxysphingosine-1,3-cycloacetal as shown by the isolation of *N*-carbobenzoxydihydrosphingosine after acid hydrolysis, as described in V, of 1.0 g. of the dihydro product; yield 0.70 g., m.p. 106–107°.

Dihydrosphingosine-1-phosphate. Erythro-2-phenyl-3-diphenylphosphoryl-4-hydroxymethyl-5-pentadecyloxazolidine, 1.50 g., in 25 ml. of 95% ethanol containing 0.4 ml. of concd. sulfuric acid was heated to boiling and allowed to stand 4 hr. at room temperature. If a precipitate formed during the interim, the solution was reheated until clear. After the addition of 35 ml. of water and subsequent cooling in an ice bath until the viscous product aggregated, the solution was centrifuged. The product was reprecipitated by dissolving it in 15 ml. of 95% ethanol with gentle warming followed by the addition of an equal volume of water with cooling. The final product obtained by centrifugation was dried over phosphorus pentoxide *in vacuo*. The combined supernatant liquids from the precipitations yielded 33.0 mg. of the dinitrophenylhydrazone of benzaldehyde; m.p. 242–244°.

The dried product, dissolved in 20 ml. of warm glacial acetic acid, was hydrogenated over 150 mg. of platinum oxide; after the cessation of hydrogen uptake, the solution was warmed and filtered with gravity, and the catalyst washed with 5 ml. of hot glacial acetic acid. To the combined filtrate and wash, after cooling to room temperature, was added 25 ml. of water. The precipitate, collected by centrifugation of the chilled solution, was washed successively (each wash being chilled) with 15 ml. of water and three times with 30-ml. portions of acetone; yield 0.77 g.; range of phosphorus values (6.89 to 7.11%).

The above product was further purified. To 247.3 mg. in 15 ml. of methanol was added 1.0 ml. of 5*N* sodium hydroxide with heating until most of the material dissolved. After the addition of 30 ml. of water and 35 ml. of ether, the solution cleared with the formation of two phases. The ether phase was discarded and the aqueous phase was re-extracted two additional times with the same volume of ether containing 10 ml. of methanol. The aqueous phase was acidified with 15 ml. of glacial acetic acid, and the precipitate, removed by centrifugation of the chilled solution, was washed with 15 ml. of water and three times with 30 ml. of acetone; yield of dihydrosphingosine-1-phosphate 94.8 mg.

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Anal. Calcd. for $C_{18}H_{40}O_5NP$ (381.0): C, 56.69; H, 10.58; N, 3.67; P, 8.13. Found: C, 56.63; H, 10.48; N, 3.58; P, 7.61.

The alkali-purified material, 97.4 mg., was oxidized with periodic acid as previously described.[10] Consumption of periodate indicated 71% completion of the reaction; yield of 2,4-dinitrophenylhydrazone of palmitaldehyde 44.0 mg., m.p. 106–107°.

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The Composition of Cigarette Smoke. V. Solanesenes

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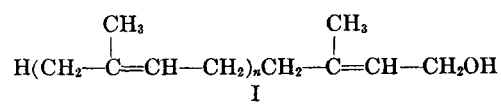
Similar series of solanesenes were obtained (a) from the cigarette smoke of a cased commercial blend of tobaccos, (b) by the dehydration of solanesol (I, n = 8), and (c) by the pyrolysis of solanesyl acetate (II, R = CH_3). Evidence is presented to indicate that a major portion of these solanesenes consists of 3-methylene-7,11,15,19,23,27,31,35-octamethyl-1,6,10,14,18,22,26,30,34-hexatriacontanonaene (III) and 3,7,11,15,19,23,27,31,35-nonamethyl-1,3,6,10,14,18,22,26,30,34-hexatriacontadecaene (IV).

In a continuation of our study of the condensable portion of cigarette smoke from a cased commercial blend of tobaccos,[1-4] a hydrocarbon fraction was isolated by chromatographic separation of a base-free hexane soluble fraction of cigarette smoke. Further chromatography yielded various phytadienes,[3] a solution of isomeric squalenes similar to that described by Kosak, *et al.*[5] and Van Duuren, *et al.*[6] in cigarette smoke plus several other fractions containing highly unsaturated aliphatic hydrocarbons. One of these latter fractions had an infrared absorption spectrum not too dissimilar from that reported for squalene regenerated from squalene hexahydrochloride using pyridine.[7] The major dissimilarity was in the presence of absorption at 6.25 μ, indicative of conjugated double bonds. Absorption at 6.01 μ and 11.92 μ (indicative of a trialkylethylene[8-11]), 6.07 μ (indicative of a terminal methylene group[8]), 7.23 μ (indicative of methyl groups[12]), 10.12 μ, 10.30 μ, 11.00 μ, 11.22 μ (indicative of the configuration $RR'C{=}CH_2$[9-11]) and 12.50 μ was noted for this fraction. This hydrocarbon fraction was sufficiently remote from the phytadiene fractions described elsewhere[3] in the chromatographic scheme to preclude contamination with these hydrocarbons as an explanation for the absorption at 6.25 μ. The elemental analysis of this material was in agreement with the empirical formula C_5H_8.

Hydrogenation of this fraction using platinum oxide as catalyst indicated ten double bonds for a molecular weight of 613 and yielded a saturated hydrocarbon whose elemental analysis, refractive index, and infrared absorption were identical with those of solanesane prepared by the catalytic hydrogenation of solanesol (I, n = 8).[13] This alcohol



I

has been reported as a constituent of flue-cured tobacco.[13] The structure originally postulated[13] for solanesol had n = 9 but this was recently re-

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