is not too surprising in view of the acidity and hydrogen bonding properties of this group, as well as the fact that the carboxyl group moment is directed at an angle to the carboxyl-ring bond and has the possibility of rotation.

Figure 7 shows a similar plot for the N_3 protons. Again, except for –COOH, the linear correlation is quite apparent, and it can be seen that the three halogens (Br, F and I) form a close cluster of points. However, in the graph for the N_1 protons, shown in Fig. 8, the F point is now well removed from the straight line. (The –COOH point will hereafter be ignored.)

However, a reasonable explanation can be offered to account for the seemingly anomalous behavior of fluorine. The lone-pair electrons of F, N_1 and the C_5–C_6 double bond form an extended conjugated system. Because of its small size, F is a more effective electron donor to the conjugated system than are the other halogens; hence it is understandable that the N_1 proton should be considerably displaced to high-field in 5-fluorouracil relative to 5-bromouracil and 5-iodouracil. (An analogous explanation accounts for the small effect of F on the acidity of benzoic acid, as compared with the other halogens.) On the other hand, N_3 is cross-conjugated with F and should thus experience a primarily inductive effect from the C_5-substituent.

Another type of correlation is shown in Fig. 9 where the average values of the two N–H shifts are plotted against Hammett σ-constants for the substituents at the 5-position. The σ-values are those for *meta* substituents, since this is the appropriate choice here. Again the –COOH point is irregular, which is not too surprising, but the linear correlation for the remaining substituents is excellent. This result again reflects the fact that the influence of 5-substituents (except–COOH) on the N–H environment follows a course predictable in advance from criteria that have proved successful in other situations.[21]

From the point of view taken in this investigation, the mobilities of the hydrogen-bonding protons

(21) E. S. Gould, "Mechanism and Structure in Organic Chemistry," Holt, Rinehart and Winston, Inc., New York, N. Y., 1960.

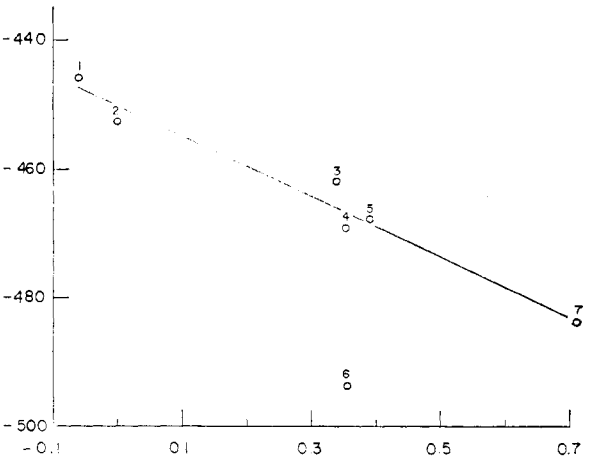

Fig. 9.—Plot of Hammett σ-constants of the substituents at the 5-position *vs.* the average values of the two amide proton shifts. The substituents are: 1, $-CH_3$; 2, –H; 3, –F; 4, –I; 5, –Br; 6, –COOH; 7, $-NO_2$. The Hammett σ-values used were calculated by Dr. C. E. Boozer. In cases where acidification was necessary before resonance frequencies became apparent this chemical shift was plotted.

appear to be the most characteristic single feature of the pyrimidine derivatives. Since 5-fluorouracil is somewhat unusual in the series for its antimetabolitic behavior, the somewhat exceptional behavior of its N_1 proton appears to be worthy of further consideration in the effort to understand its role in biological systems.

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We wish to thank Hoffmann-LaRoche, Inc., Nutley 10, N. J., for supplying us with the sample of fluorouracil used in this study.

[CONTRIBUTION FROM THE DANIEL SIEFF RESEARCH INSTITUTE, THE WEIZMANN INSTITUTE OF SCIENCE, REHOVOTH, ISRAEL]

Studies on Sphingolipids. VII. Synthesis and Configuration of Natural Sphingomyelins[1]

BY DAVID SHAPIRO AND H. M. FLOWERS

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Both enantiomorphs of *erythro*-sphingomyelin have been synthesized. The synthesis involves acid-catalyzed ring scission of the suitably substituted *cis*-oxazoline I and the optical resolution of II. Acylation of the latter and phosphorylation of III with β-chloroethylphosphoryl dichloride followed by treatment of the β-chloroethylphosphates of 3-O-benzoylceramides (IV) with trimethylamine leads to the sphingomyelins (VI). DL-*threo*-Dihydrosphingomyelin has been prepared similarly from the *trans*-oxazoline I. Natural sphingomyelin has been found identical with the D-*erythro*-enantiomorph.

The generally accepted view that the asymmetric carbon atoms in sphingomyelin have the *erythro* configuration is largely based on analogy with the cerebrosides whose structure has been firmly established.[2,3] However, the surprising finding

(1) Supported in part by a grant from Mr. Samuel Rothberg of Peoria, Ill.

(2) H. E. Carter and Y. Fujino, *J. Biol. Chem.*, **221**, 879 (1956).

(3) D. Shapiro and H. M. Flowers, *J. Am. Chem. Soc.*, **83**, 3327 (1961).

TABLE I

β-CHLOROETHYLPHOSPHATES OF 3-O-BENZOYLCERAMIDES (IV)

IV	Base, sphingosine	N-Acyl	M.p., °C.	$[\alpha]^{25}D$ (chlf.)	Formula	Analyses, % Calcd. C	H	N	P	Cl	Found C	H	N	P	Cl
A	D-*erythro*-	Palmitoyl	76–78	+3.6°	$C_{43}H_{75}NO_7PCl$	65.83	9.65	1.78	3.95	4.52	65.69	9.76	1.99	3.75	4.69
B	D-*erythro*-	Lignoceroyl	79–81	+5.4	$C_{61}H_{91}NO_7PCl$	68.31	10.23		3.45	3.95	68.54	10.24		3.25	3.71
C	D-*erythro*-	Stearoyl	81–83	+4.8	$C_{45}H_{79}NO_7PCl$	66.52	9.80		3.81	4.37	66.85	9.94		3.68	3.97
D	L-*erythro*-	Palmitoyl	77–78	−4.2	$C_{43}H_{75}NO_7PCl$	65.83	9.65	1.78	3.95	4.52	65.52	9.44	2.05	3.61	4.95
E	D-*erythro*-dihydro-	Palmitoyl	105–106	0	$C_{43}H_{77}NO_7PCl$	65.65	9.86		3.94	4.51	65.28	9.75		3.43	4.3
	D-*erythro*-dihydro- (Ba salt)		95–98	−2	$C_{86}H_{152}N_2O_{14}P_2Cl_2Ba$	60.40	9.07	1.64	3.62	4.15	60.17	9.22	1.92	3.16	4.04
G	DL-*threo*-dihydro-	Palmitoyl	66–68	...	$C_{43}H_{77}NO_7PCl$	65.65	9.86		3.94	4.51	65.70	9.95		3.99	4.77

TABLE II

SYNTHETIC SPHINGOMYELINS (VI, A-G)

VI	Base, sphingosine	N-Acyl	M.p., °C.	$[\alpha]^{25}D$ (chlf.:MeOH), 1:1	Formula	Analyses, % Calcd. C	H	N	P	Found C	H	N	P
A	D-*erythro*-	Palmitoyl	215–217	+6.1°	$C_{39}H_{81}N_2O_7P$	64.96	11.32	3.89	4.30	65.01	11.30	3.90	4.18
B	D-*erythro*-	Lignoceroyl	213–214	+6.5	$C_{47}H_{97}N_2O_7P$	67.75	11.66	3.36	3.70	67.62	11.56	3.51	3.82
C	D-*erythro*-	Stearoyl	213–214	+6.1	$C_{41}H_{85}N_2O_7P$	65.69	11.44	3.74	4.13	65.99	11.60	3.30	3.71
D	L-*erythro*-	Palmitoyl	216–217	−6.5	$C_{39}H_{81}N_2O_7P$	64.96	11.32	3.89	4.30	64.30	11.52	3.86	4.15
E	D-*erythro*-dihydro-	Palmitoyl	222–223	+22.5	$C_{39}H_{83}N_2O_7P$	64.78	11.58	3.87	4.29	64.38	11.62	3.72	3.96
F	D-*erythro*-dihydro-	Stearoyl[a]	221–222	+20.5	$C_{41}H_{87}N_2O_7P$	65.56	11.67	3.73	4.12	66.0	11.40	3.44	3.55
G	DL-*threo*-dihydro-	Palmitoyl	224–225	....	$C_{39}H_{83}N_2O_7P$	64.78	11.58	3.87	4.29	64.62	11.74	3.58	4.05

[a] Obtained by hydrogenation of VIc with platinum oxide.

of Sribney and Kennedy[4] that the enzymatic synthesis of sphingomyelin requires the *threo* rather than the *erythro* form of the sphingosine moiety has aroused some uncertainty as to the correctness of this assumption. It was considered desirable, therefore, to provide direct chemical proof for the accepted structure. A quantitative degradation of sphingomyelins by mild acid or alkali is difficult to achieve, while more drastic conditions cause considerable epimerization. Accordingly, we investigated the synthetic approach with the objective of preparing the four possible optical isomers of sphingomyelin.

In two previous papers[5,6] we reported syntheses of racemic sphingomyelin and dihydrosphingomyelin by a route which involved phosphorylation of the substituted oxazoline I with β-chloroethylphosphoryl dichloride and subsequent ring opening of the phosphate ester at the position 2–3. Acylation of the resulting free amino group led to the racemic compound IV. However, it was found that this method is not applicable to a total synthesis. The optical resolution of I failed because of both the instability of its salts and the sensitivity of the ring system toward resolving agents such as bromocamphorsulfonic acid. Likewise unsuccessful were preliminary trials to bring about the optical resolution of IV.

I,a,b — II,a,b — III,a,b — IV (Table I) — V — VI, A-G (Table II)

In a recent communication[3] we have shown that the *cis*-oxazoline I is converted into the *erythro*-benzoate IIa,b by treatment with dilute sulfuric acid in tetrahydrofuran. The pure sulfate obtained in quantitative yield could be easily resolved by means of barium D- or L-tartrate. Surprisingly, it was now found that the *threo*-diastereomer IIb showed a different behavior. Our continuous attempts to resolve this isomer by the same method were unsuccessful. We were unable to obtain a tartrate with constant physical properties,

(4) M. Sribney and E. P. Kennedy, *J. Biol. Chem.*, **233**, 1315 (1958).

(5) D. Shapiro, H. M. Flowers and S. Spector-Shefer, *J. Am. Chem. Soc.*, **81**, 3743 (1959).

(6) D. Shapiro, H. M. Flowers and S. Spector-Shefer, *ibid.*, **81**, 4360 (1959).

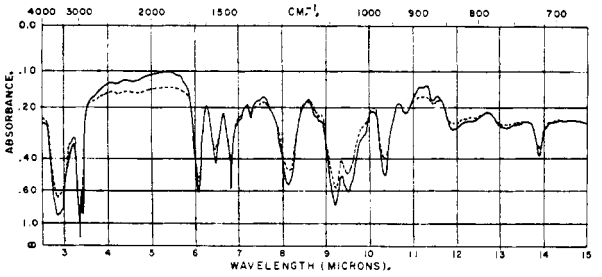

Fig. 1.—Infrared spectra of sphingomyelins (in KBr): solid line, synthetic D-*erythro*-sphingomyelin (VI, C); dashed line, natural sphingomyelin.

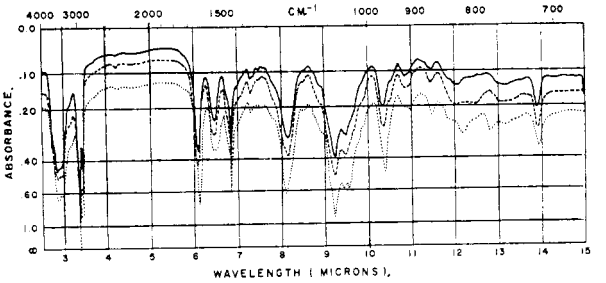

Fig. 2.—Infrared spectra of dihydrosphingomyelins (in KBr). The absorption peaks in the region of 9–13 μ are: synthetic D-*erythro*-(VI, F, solid line[a] and natural (dashed line), 9.48, 10.84, 12.04 and 13.1 μ; synthetic DL-*threo*-(VI, G, dotted line), 9.40, 9.54, 10.96, 12.14, and 12.80 μ.

[a] The racemic compound corresponding to VI, E gave a similar curve.

and it appeared that some changes took place in the molecule during repeated recrystallization from alcohol. This divergence suggests the existence of a steric effect inherent in the *threo* relationship of the carbon atoms 2 and 3 in II.

Both the sulfates and the optically active tartrates of II were acylated in the presence of sodium acetate and acetic acid to give high yields of the ceramides III with the secondary hydroxyl blocked by a benzoyl group. The substituted ceramides were phosphorylated with β-chloroethylphosphoryl dichloride and the resulting purified phosphate ersters IV (Table I) were obtained in yields of 50–60%. The continuation of the synthesis proceeded in the manner described for the racemic lipids, except that the quaternary chlorides V were not isolated. The present route was found superior to the method previously reported in that it gives better yields, the over-all yield IV→VI being about 40%. Furthermore, it excludes the possibility of an undesirable ring scission which may occur during phosphorylation of I, and gives products of higher purity. Thus, N-palmitoyl-DL-dihydrosphingomyelin had a melting point of 218–219°, as compared with 210–212° observed previously.[5]

The synthetic sphingomyelins VI, A-G, are slightly hygroscopic powders, with elemental analyses conforming with the "hydrated" rather than with the zwitterionic form even after prolonged drying in high vacuum at 80°. Table II shows that the fatty acid constituent is an exiguous factor in their physical properties. This fact, also observed in the cerebrosides series, renders possible a comparison of the synthetic products with the natural lipids. A sample of natural sphingomyelin with a melting point of 213–214° and a specific rotation of +6.9° was prepared from bovine spinal cord[7] following the procedure of Carter, *et al.*,[8] as modified by Marinetti and Stotz.[9] The dihydro derivative melted at 222–223° and had a specific rotation of +21.7°. This characteristic increase in dextrorotation as a result of hydrogenation was also observed with the synthetic products. As can be seen in Table II, the physical properties of the D-*erythro*-sphingomyelins (VI,A-C) and of the dihydro derivatives (VI,E-F) are in good agreement with those of the respective natural products. It is noteworthy that the optical rotation of −6.5° for the L-isomer (VID) indicates the satisfactory separation of the enantiomorphs.

The close similarity of the infrared spectra of the synthetic *erythro* compounds with those of the natural lipids is shown in Figs. 1 and 2. On the other hand, significant differences were found in the spectrum of DL-*threo*-dihydrosphingomyelin (VI, G) in the region of 9–13 μ where several bands were shifted. As can be seen in Fig. 2, the most conspicuous divergence is a split of the band at 9.48 μ into a doublet at 9.40 and 9.54 μ.

Taken together, the above results provide conclusive evidence that the natural sphingomyelins have indeed the D-*erythro* configuration.

Experimental

3-O-Benzoylceramides (III) were prepared by the method recently described for similar derivatives of sphingosine.[3]

erythro-**N-Palmitoyl-3-O-benzoyl-D-sphingosine**, m.p. 88–90°, $[\alpha]^{25}D$ +13.0° (*c* 1.05, $CHCl_3$). *Anal.* Calcd. for $C_{41}H_{71}NO_4$: C, 76.68; H, 11.14; N, 2.18. Found: C 76.27; H, 11.11; N, 2.03.

erythro-**N-Stearoyl-3-O-benzoyl-D-sphingosine**, m.p. 86–88°, $[\alpha]^{25}D$ +16.7° (*c* 1.5, $CHCl_3$). *Anal.* Calcd. for $C_{43}H_{75}NO_4$: C, 77.08; H, 11.29; N, 2.09. Found: C, 77.22; H, 11.10; N, 2.23.

erythro-**N-Palmitoyl-3-O-benzoyl-L-sphingosine**, m.p. 87–89°, $[\alpha]^{25}D$ −12.6° (*c* 2.28, $CHCl_3$). *Anal.* Calcd. for $C_{41}H_{71}NO_4$: C, 76.68; H, 11.14; N, 2.18. Found: C, 76.41; H, 11.30; N, 1.92.

erythro-**N-Palmitoyl-3-O-benzoyl-D-dihydrosphingosine**, m.p. 78–80°, $[\alpha]^{25}D$ +26.4° (*c* 1.25, $CHCl_3$). *Anal.* Calcd. for $C_{41}H_{73}NO_4$: C, 76.46; H, 11.42; N, 2.18. Found: C, 76.81; H, 11.41; N, 2.13.

threo-**N-Palmitoyl-3-O-benzoyl-DL-dihydrosphingosine**, was prepared from the *trans*-oxazoline I; m.p. 62–64°. *Anal.* Calcd. for $C_{41}H_{73}NO_4$: C, 76.46; H, 11.42; N, 2.18. Found: C, 76.52; H, 11.33; N, 2.27.

***trans*-2-Phenyl-4-hydroxymethyl-5-pentadecyl-2-oxazoline** (I).—Condensation of *threo*-methyl 2-amino-3-hydroxystearate[6] with ethyl iminobenzoate in chloroform solution and subsequent reduction[6] with lithium aluminum hydride afforded the *trans*-oxazoline in 71% over-all yield, m.p. 83–85°.

Anal. Calcd. for $C_{25}H_{41}O_2N$: C, 77.47; H, 10.67; N, 3.61. Found: C, 77.43; H, 10.58; N, 3.78.

β-Chloroethylphosphates of 3-O-Benzoylceramides (IV).—To a stirred solution of β-chloroethylphosphoryl dichloride[10,11] (0.0066 mole) in dry chloroform (10 ml.) cooled to −10° was added dropwise dry pyridine (0.0066 mole).

(7) Supplied by Armour Pharmaceutical Co., Kankakee, Ill.

(8) H. E. Carter, W. J. Haines, W. E. Ledyard and W. P. Norris, *J. Biol. Chem.*, **169**, 77 (1947).

(9) G. Marinetti and E. Stotz, *J. Am. Chem. Soc.*, **76**, 1347 (1954).

(10) R. R. Renshaw and C. Y. Hopkins, *ibid.*, **51**, 953 (1929).

(11) Freshly prepared chloride is absolutely essential.

After the addition of the ceramide III (0.0066 mole) dissolved in dry chloroform (20 ml.) had been completed in 5–10 minutes, the temperature was maintained at 0– +5° for 4–5 hours. The clear reaction mixture was transferred to a vigorously stirred solution of 5% barium hydroxide (in slight excess), and the temperature was allowed to rise slowly to 20°. After 30 minutes, the mixture was recooled to 10° and cold ether was added slowly. Stirring was continued for 1 hour at a final temperature of 20–25°. The upper layer was separated, washed several times with water and left overnight in the refrigerator. The bulky precipitate which separated was washed with a little cold acetone and ether and air-dried. Owing to the greater solubility of the unsaturated derivatives, it was found advantageous in this case to cool the solution to −10° for about 30 minutes before filtering off the precipitated barium salt.

The barium salts isolated were decomposed by shaking with a mixture of ether and dilute hydrochloric acid, and the residue obtained on evaporation of the ether solution was crystallized from methanol at room temperature, giving 50–60% yields of the β-chloroethylphosphates; infrared spectra: significant bands at 5.8 and 7.9 μ (benzoate), 6.0 and 6.6 μ (amide), 7.9, 10.3 and 11.5 μ (phosphate), 10.3 μ (*trans*-ethylenic —C=C—) 9.05, 9.15, 9.65, 9.75 μ.

Quaternization.—The conversion of the free phosphoric acids to the pure barium salts and subsequent preparation of the sphingomyelins was carried out by the previous method,[5,6] and the pure materials were isolated in yields of 60–70%.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX S. A., MEXICO, D. F., MEX.]

Steroids. CLXXXVIII.[1] New Fluorination Procedures. Part 3.[2] *cis*-Addition of Fluorine to a Steroid Olefin. A New Route to 6α-Fluoro-Δ⁴-3-ketones[3]

BY A. BOWERS, P. G. HOLTON, E. DENOT, M. C. LOZA AND R. URQUIZA

RECEIVED OCTOBER 19, 1961

An *in situ* preparation of lead tetrafluoride from lead tetraacetate and anhydrous hydrogen fluoride has been utilized for the controlled fluorination of pregnenolone and pregnenolone acetate. *cis*-Addition of fluorine takes place to afford the corresponding 5α,6α-difluoro analogs. Their ready conversion into 6α-fluoroprogesterone represents a new route to the biologically important 6α-fluoro-Δ⁴-3-ketones. The addition of hydrogen fluoride to pregnenolone acetate and the reaction between hydrogen fluoride and pregnenolone are described.

It has recently been demonstrated that introduction of a 6α-fluoro substituent[4–15] to a series of steroid hormones favorably influenced biological activity. Previous approaches to these compounds had proceeded *via* (a) fission of a 5α,6α-epoxide with boron trifluoride etherate[4,5,7–11,13–16] or anhydrous hydrogen fluoride,[6,12,15] (b) *trans*-addition of BrF to a Δ⁵-3β-alcohol[17] or (c) perchloryl fluoride treatment of the derived enol ether[18] or enol acetate[19] of a Δ⁴-3-ketone.

All of these approaches afforded 6α-fluoro-Δ⁴-3-ketones primarily through their 6β-fluoro epimers. The direct introduction of a 6α-fluorine atom is now described *via* the *cis*-addition of fluorine to a Δ⁵-olefin.

The addition of fluorine to carbon–carbon double bonds has found very little application because of the extremely vigorous and uncontrolled nature of the interaction of fluorine with both carbon–carbon and carbon–hydrogen bonds. Even dilution of fluorine with nitrogen or carbon dioxide and carrying out the reaction at low temperatures has had only very limited success.[20] Only in perhalogenated olefins has it been possible to effect the addition of fluorine to a double bond.[21] However in many instances chlorofluoroalkenes are fluorinated with addition, substitution and disproportionation taking place.[22] The controlled addition of fluorine to olefins thus posed an interesting problem.

An attractive approach appeared to be in a study of the potential of lead tetrafluoride for reactions of this type. In 1947, lead tetrafluoride was reported to be unsatisfactory as a fluorinating agent for hydrocarbons in the vapor phase,[23] but slightly prior to this work Henne and Waalkes showed that an *in situ* preparation of PbF_4 from PbO_2 and

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