

H, 2.80; N, 6.26; S, 13.55; mol. wt.,  $1539 \pm 200$  (cryoscopic in *m*-dinitrobenzene).

**Conversion of the Polythioester II to L-Cystine.**—Polythioester II (0.2 g.) was heated for 24 hours under reflux with 2 ml. of hydriodic acid and 2 ml. of glacial acetic acid. The insoluble part was removed by filtration and the filtrate evaporated under reduced pressure to dryness. The residue was dissolved in 3 ml. of water, extracted with ether and adjusted to pH 4.5 with a saturated sodium acetate solution. After several days L-cystine separated, m.p.  $252^\circ$  dec.,  $[\alpha]_{20}^D -187^\circ$  (*c* 0.16 in *N* HCl). The compound was in all properties identical with an authentic specimen of L-cystine.

*Anal.* Calcd. for  $C_6H_{12}N_2O_4S_2$ : C, 30.00; H, 5.04; N, 11.64. Found: C, 30.30; H, 4.75; N, 11.93.

The ether extract was evaporated and the residue sublimed *in vacuo* to give 49 mg. of phthalic anhydride, m.p. and mixed m.p.  $130^\circ$ .

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

## II. Synthesis of Long Chain Fatty Acid Amines of Sphingosine and Dihydrosphingosine<sup>1</sup>

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The acyl chlorides of the even numbered fatty acids from C-12 to C-18 were allowed to react with either sphingosine or dihydrosphingosine in *N,N*-dimethylformamide and pyridine to yield the corresponding *N*-acyl derivatives. Reduction of *N*-stearoylsphingosine and *N*-stearoyldihydrosphingosine with lithium aluminum hydride gave the respective secondary amines; the olefinic bond of sphingosine in the former compound was unaffected by the reduction.

In continuation of our program of preparing various sphingosine and dihydrosphingosine derivatives for substrates in our forthcoming studies on the *in vitro* metabolism of cerebroside and sphingomyelin, it was necessary to have a selection of long chain fatty acid amides of sphingosine (ceramides) and dihydrosphingosine (dihydroceramides). The ceramide *N*-lignocerylsphingosine was first isolated from partially hydrolyzed sphingolipide preparations<sup>2,3</sup> and, later, was found free in both liver and spleen<sup>4-6</sup>; *N*-cerebronylsphingosine was prepared by partial hydrolysis of phrenosine.<sup>7</sup> Reichel and Thannhauser,<sup>8</sup> *via* an indirect route, synthesized *N*-stearoyl- and *N*-palmitoylsphingosine, by first preparing the triacylated derivative, *e. g.*, tristearoylsphingosine, and then selectively hydrolyzing the ester groups to obtain the *N*-acyl compound. Unsatisfactory results were obtained by us with this procedure, owing primarily to the intractable emulsions formed in the separation of the free fatty acid from the ceramide and the resulting low yields. An alternative method involving less preparative steps was sought in which the amine function was treated with the appropriate acyl chloride to give directly the *N*-substituted compound. A study of various solvents disclosed that of those examined, *N,N*-dimethylformamide was the most suitable since it appeared to suppress the nucleophilic property of the hydroxyl group while

enhancing that of the amino group. This solvent had been used in the preparation of the *N*-palmitoyl derivative of chloramphenicol.<sup>9</sup> In the initial experiments, one-half the stoichiometric amount of acyl chloride was treated with sphingosine or dihydrosphingosine in order to obviate the difficulty of separating unreacted fatty acid from the product; the excess base served to neutralize the acid formed. Since this was wasteful of valuable sphingosine, pyridine was added to the reaction mixture in amounts sufficient to act as acid acceptor. When 90% of the stoichiometric amount of acyl chloride was employed with either sphingosine or dihydrosphingosine in the presence of pyridine, the yield was correspondingly increased and the product was identical with that obtained in the absence of pyridine. The ceramides and dihydroceramides formed from the C-16 and C-18 acyl chlorides were the least difficult to prepare since they precipitated out of the reaction mixture thus facilitating their isolation and purification. However, since little or no precipitate occurred with either the C-12, C-14 or oleoyl compounds, the cold reaction mixture had to be acidified, extracted with ether, and the residue, obtained after washing and concentrating the ether solution, crystallized from 95% ethanol. The *N*-acylsphingosine and *N*-acyldihydrosphingosine derivatives gave negative ester<sup>10</sup> and ninhydrin reactions. The melting points in the homologous series of dihydroceramides showed an increase with chain length of the acyl substituent; lauroyl,  $99-101^\circ$ ; myristoyl,  $103-104^\circ$ ; palmitoyl,  $105-106^\circ$ ; and stearoyl,  $106-107^\circ$ . The same was true with the sphingosine homologs which had lower melting points than their corresponding saturated derivatives: lauroyl,  $80-82^\circ$ ;

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TABLE I

N-Acylsphingosine	Empirical formula	Yield, g.	M.p., °C.	Carbon, %		Hydrogen, %		Nitrogen, %	
				Calcd.	Found	Calcd.	Found	Calcd.	Found
Stearoyl	C <sub>36</sub> H <sub>71</sub> O <sub>3</sub> N	1.31	89-91	76.38	75.97	12.65	12.81	2.47	2.45
Palmitoyl	C <sub>34</sub> H <sub>67</sub> O <sub>3</sub> N	1.35	89-91	75.90	75.78	12.56	12.73	2.60	2.56
<i>n</i> -Octadecylsphingosine	C <sub>36</sub> H <sub>73</sub> O <sub>2</sub> N	0.71	59-61	78.32	78.04	13.34	13.59	2.54	2.53
N-Acyldihydrosphingosine									
Stearoyl	C <sub>36</sub> H <sub>73</sub> O <sub>2</sub> N	1.78	106-107	76.11	76.38	12.97	13.03	2.47	2.47
Palmitoyl	C <sub>34</sub> H <sub>69</sub> O <sub>2</sub> N	1.99	105-106	75.61	75.70	12.89	13.00	2.59	2.54
<i>n</i> -Octadecyldihydrosphingosine	C <sub>36</sub> H <sub>76</sub> O <sub>2</sub> N	0.83	73-75	78.03	77.88	13.66	13.55	2.53	2.47
Phenylthiourea deriv. of <i>n</i> -octadecyldihydrosphingosine	C <sub>43</sub> H <sub>80</sub> O <sub>2</sub> N <sub>2</sub> S		83-85	74.92	75.28	11.71	-11.88	4.07	4.06

myristoyl, 84-87°; palmitoyl, 89-91°; and stearoyl, 89-91°. N-Oleoylsphingosine (m.p. 77-80°) and N-oleoyldihydrosphingosine (m.p. 96-99°), each of which melted in the range of the respective N-lauroyl compound, were converted easily to N-stearoyldihydrosphingosine by hydrogenation over platinum oxide.

In order to prepare the amine from the long chain fatty acid amide, N-stearoyldihydrosphingosine was refluxed with lithium aluminum hydride to yield N-*n*-octadecyldihydrosphingosine which was further characterized as the phenylthiourea derivative. Reduction of N-stearoylsphingosine gave the corresponding N-*n*-octadecylsphingosine in which the double bond of sphingosine remained intact.

### Experimental

Sphingosine sulfate was isolated from beef spinal cord lipids as previously described.<sup>11</sup> The acyl chlorides were prepared by refluxing for two hours 0.5 mole of fatty acid with 0.8 mole of thionyl chloride in 125 ml. of carbon tetrachloride. The reaction mixture was concentrated on the water-pump to remove the excess thionyl chloride and carbon tetrachloride and then distilled under vacuum to yield the acyl chloride. The thionyl chloride was distilled over linseed oil prior to the preparation of oleoyl chloride.<sup>12</sup>

**N-Acylsphingosine (I).**—Sphingosine sulfate (14.0 g.) was dissolved in 300 ml. of ether and 100 ml. of 0.5 *N* NaOH with gentle warming. The aqueous layer was re-extracted with 100 ml. of ether and the combined ether extracts, after being washed with 100 ml. of water, were concentrated under reduced pressure. The residue was successively dried over phosphorus pentoxide, crystallized from 300 ml. of *n*-heptane and stored *in vacuo* over paraffin and phosphorus pentoxide; yield of free base, 10.4 g.

To 3.0 g. of the base (10 mmoles, dissolved in 40 ml. of *N,N*-dimethylformamide with mild heating, were added 1 ml. of dry pyridine and 9.0 millimoles of the acyl chloride in 10 ml. of *N,N*-dimethylformamide. After standing at room temperature one hour, the reaction mixture was stored in the cold at 5° overnight. The precipitate was removed by suction filtration and crystallized with the aid of Norite from 95% ethanol (100 ml. per g.); a second crystallization from absolute ethanol (75 ml. per g.) yielded the pure product (Table I). The products gave negative ester and ninhydrin reactions.

**N-Acyldihydrosphingosine (II).**—The free base sphingosine (6.0 g.), obtained by ether extraction of the sulfate from alkaline solution as described in I and without prior drying over phosphorus pentoxide, was reduced over 300 mg. of platinum oxide in 100 ml. of ethanol-ethyl acetate (1:1). When the uptake of hydrogen ceased, the reaction mixture was warmed and filtered with suction. The residue, obtained by concentrating the filtrate under diminished pressure, was crystallized from 200 ml. of *n*-heptane and the product was stored as was the unsaturated base in I; yield of dihydrosphingosine, 5.1 g.

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Dihydrosphingosine (2.0 g., 6.6 mmole) was treated with 6.0 millimoles of acyl chloride according to the procedure employed in the preparation of I. The product was removed from the reaction mixture by filtration and crystallized from absolute ethanol (100 ml. per g.) (Table I).

**Reduction of N-Oleoylsphingosine and N-Oleoyldihydrosphingosine (III).**—N-Oleoylsphingosine (200 mg.) was hydrogenated over 150 mg. of platinum oxide in 50 ml. of ethanol-ethyl acetate (1:1). Following the reduction, the reaction mixture was filtered and concentrated, and the residue crystallized from 50 ml. of absolute ethanol; yield 150 mg., m.p. 105-106°; 200 mg. of N-oleoyldihydrosphingosine subjected to the same procedure yielded 195 mg. of N-stearoyldihydrosphingosine, m.p. 105-106°.

**Conversion of N-Stearoylsphingosine to N-*n*-Octadecylsphingosine (IV).**—N-Stearoylsphingosine (1.0 g.), suspended in 60 ml. of anhydrous ether, was refluxed for 3.0 hours with 5 ml. of a 2 molar slurry of lithium aluminum hydride in ether. The excess hydride was decomposed with methanol and the base was extracted with 300 ml. of ether after the addition of 100 ml. of 2 *N* KOH. The ether extract was washed with two 50-ml. portions of water, filtered to remove the last traces of aluminum salts, concentrated, and, finally, crystallized from 50 ml. of *n*-heptane after drying *in vacuo* over phosphorus pentoxide; yield 0.71 g., m.p. 59-61° (Table I). The iodine number<sup>13</sup> was 71% of theory (46.0 calcd., 32.6 found) which is in the range found for other substituted sphingosines: N-carbobenzoxysphingosine 58.7 calcd., 37.9 found; N-trifluoroacetylsphingosine 64.3 calcd., 39.7 found; 3-O-methyl-N-carbobenzoxysphingosine 56.8 calcd., 39.0 found. These observations are in accordance with those found by Carter, *et al.*,<sup>14</sup> for other substituted sphingosines in which the values obtained were less than theory. Hydrogenation of 100 mg. of N-*n*-octadecylsphingosine over 100 mg. of platinum oxide in 50 ml. of ethanol-ethyl acetate (1:1) yielded 80 mg. of N-*n*-octadecyldihydrosphingosine (V), m.p. 73-75°.

**Conversion of N-Stearoyldihydrosphingosine to N-*n*-Octadecyldihydrosphingosine (V).**—N-Stearoyldihydrosphingosine (1.0 g.) was treated with lithium aluminum hydride according to the procedure described for N-stearoylsphingosine in IV. The residue obtained after removal of the ether was crystallized from 100 ml. of *n*-heptane; yield 0.83 g., m.p. 73-75° (Table I). N-*n*-Octadecyldihydrosphingosine, upon reaction with phenyl isothiocyanate in the usual manner, yielded the corresponding phenylthiourea derivative, m.p. 83-85° (Table I); efforts to obtain the tosyl derivative were unsuccessful. A 2% solution of N-*n*-octadecyldihydrosphingosine in chloroform showed no observable optical activity.

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