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SYNTHETIC STUDIES ON SPHINGOLIPIDS. XI. SYNTHESIS OF CYTOLIPIN H AND ANALOGOUS LACTOSIDES**

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We report the synthesis of natural cytolipin H and its saturated analogues. The procedure involves condensation of 3-0-substituted ceramides with heptaacetyl lactosyl bromide in the presence of mercuric cyanide. The analogues include: (a) ceramide lactosides with a homologous series of fatty acids from C_{18} to C_2; (b) lactosides derived from *allo*-threoninol, threoninol and serinol.

We also describe the synthesis of lactosyl sphingosine and its saturated diastereoisomer.

Cytolipin H, first isolated from human epidermoid carcinoma[1]), has been identified[2]) as a β-lactoside of N-acylsphingosine (IIIa, R'CO = lignoceroyl). Further investigations by Rapport and his co-workers[3]) would seem to indicate that the immunological specificity of this hapten is largely determined by the carbohydrate portion. However, for lack of material it has not been possible to ascertain the specific role of the hydrophobic part of the molecule, namely to determine the effect of variation of chain length of both the fatty acid and the sphingosine base on the serological activity. In an effort to make available such compounds we have undertaken the synthesis of a series of cytolipin H analogues with the fatty acids decreasing from C_{18} to C_2. Since the absence of the double bond in the sphingosine moiety of the lactoside does not seem to affect its serological activity[4]), we were contented with the preparation of the more readily accessible dihydro derivatives. In order to obtain information on the specificity of the basic part of the molecule, we have synthesized the analogous lactosides of *allo*-threoninol, threoninol and serinol which are the lowest homologues of sphingosine.

In a recent preliminary communication[5]) reporting the synthesis of natural cytolipin H and its racemic form we pointed out that in order to assure an unequivocal synthesis of a sphingolipid involving substitution at carbon 1

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of a ceramide (N-acylsphingosine), it is necessary to block the secondary hydroxyl group. Consequently, we employed in the present investigation the disubstituted bases II which may be conveniently prepared by acylation of the 3-O-benzoate I[7,8]). The aglucons II (table 1) were condensed with hepta-acetyl-α-D-lactosyl bromide in a mixture of nitromethane and benzene in the presence of mercuric cyanide, under carefully controlled conditions. The crude heptaacetyl derivatives of III were separated by a silica gel column and, in most cases, directly treated with methanolic sodium methylate. The resulting deacylated lactosides were first purified by a silicic acid column and then crystallized from either pyridine acetone or chloroform-methanol.

In order to test the effect of configuration of the sphingosine base in the cytolipin H molecule on the immunological activity, we have also synthesized the *threo* form of IIIb (R'CO = lignoceroyl). The required *threo* diastereoisomer of Ib resulted from an acid-catalyzed scission of the *trans*-oxazoline VIIIa obtained by reduction of VII. The latter compound arose from the *erythro* form of VIa by a ring closure accompanied by a Walden inversion[8]). As starting material we employed the amino ester V[6]).

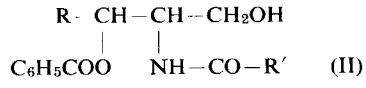
In the synthesis of mono-lactosides of the lower trifunctional amino-alcohols we followed the principle applied to the sphingosine series which involves a suitable protection by an oxazoline system. Thus, for the preparation of the benzoyl derivatives of threoninol, *allo*-threoninol and serinol (Ic, d) we employed the oxazolines VIIIb, c. The intermediates VII were obtained by two different routes following the method of Elliott[9]). The threonine and serine esters (Vb, c) were condensed with ethyl iminobenzoate directly to VII, while the *allo*-threonine derivative was prepared via the benzamido ester VIb, as outlined in scheme 2.

The physical properties of the lactosides are listed in table 2. They crystallize with one molecule of water and are slightly soluble in methanol and acetone, but soluble in pyridine and chloroform. The solubility of the lower members of this series increases in methanol and decreases in chloroform. The D- and DL-ceramide lactosides (IIIa, R'CO = lignoceroyl), were examined by Rapport and Graf by the complement-fixation technic[10]) and found identical with natural cytolipin H in their serological activity. All cytolipin H analogues were shown by thin-layer chromatography to be homogeneous compounds. The infrared spectra exhibited a maximum at 11.2 μ[11-13]), indicating the presence of a β-glycoside, which is consistent with the previously observed specificity of the glycosidation method applied[6]).

Recently, Weinberg *et al.*[14]) have published a synthesis of N-palmitoyl-sphingosyl lactoside employing silver oxide as condensing agent. However, they reported a melting point of 108° (pure ceramide lactosides were found to melt in the range of 230–240°) and a specific rotation of +8.1°, as com-

TABLE 1

3—O—Benzoyl-ceramides,



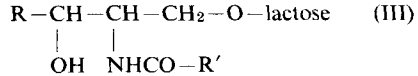
(II)

R	Parent base	R′	m.p. (°C)	Formula	Calculated			Found		
					C	H	N	C	H	N
$CH_3(CH_2)_{14}$	DL-Dihydro-sphingosine (*erythro*)	$CH_3(CH_2)_{12}$	72–73	$C_{39}H_{69}NO_4$	76.05	11.30	2.27	76.28	11.21	2.58
		$CH_3(CH_2)_{10}$	71–72	$C_{37}H_{65}NO_4$	75.59	11.14	2.38	75.56	11.21	2.14
		$CH_3(CH_2)_8$	69–70	$C_{35}H_{61}NO_4$	75.08	10.98	2.50	74.90	10.80	2.78
		$CH_3(CH_2)_6$	56–57	$C_{33}H_{57}NO_4$	74.53	10.80	2.63	74.49	10.90	2.79
		$CH_3(CH_2)_4$	51–52	$C_{31}H_{53}NO_4$	73.91	10.61	2.78	73.70	10.60	2.90
		$CH_3(CH_2)_2$	54	$C_{29}H_{49}NO_4$	73.22	10.38	2.94	73.24	10.38	3.18
	DL-Dihydro-sphingosine (*threo*)	$CH_3(CH_2)_{22}$	65	$C_{49}H_{89}NO_4$	77.81	11.86	1.85	77.59	11.64	1.81
		Cl_2CH	66–67	$C_{27}H_{43}NO_4Cl_2$ [a]	62.78	8.39	2.71	63.13	8.47	2.92
CH_3	DL-*Allo*-threoninol	$CH_3(CH_2)_{22}$	90	$C_{35}H_{61}NO_4$	75.1	10.91	2.5	75.13	11.07	2.46
	DL-Threoninol	$CH_3(CH_2)_{22}$	98–100	$C_{35}H_{61}NO_4$	75.1	10.91	2.5	74.73	10.70	2.56
H	DL-Serinol	$CH_3(CH_2)_{22}$	81–82	$C_{34}H_{59}NO_4$	74.81	10.90	2.57	75.28	10.94	2.31

[a] Cl, calculated 13.73. Found 13.46.

TABLE 2

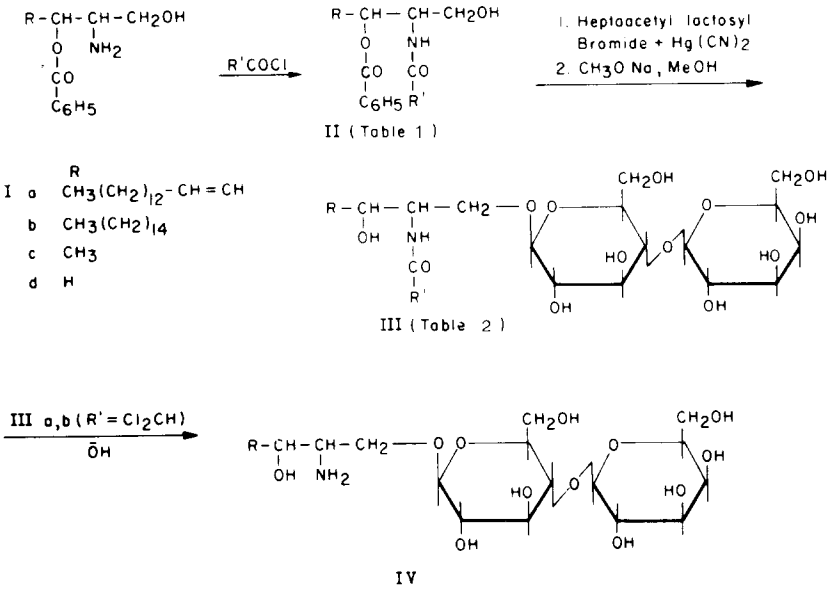
Lactosyl-ceramides,



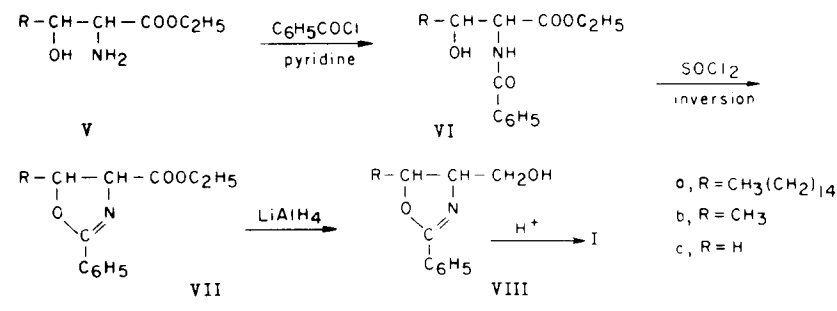
(III)

R	Parent base	R′	m.p. (°C)	$[\alpha]_D^{25}$ (pyridine)	Formula	Calculated C	Calculated H	Found C	Found H
$CH_3(CH_2)_{12}-CH=CH$	D-Sphingosine (*erythro*)	$CH_3(CH_2)_{22}$ [a]	235	−6.6°	$C_{54}H_{103}NO_{13}.H_2O$	65.34	10.67	64.79	10.54
	DL-Sphingosine (*erythro*)	$CH_3(CH_2)_{22}$ [a]	235	−4°	$C_{54}H_{103}NO_{13}.H_2O$	65.34	10.67	65.47	10.98
$CH_3(CH_2)_{14}$	DL-Dihydro-sphingosine(*threo*)	$CH_3(CH_2)_{22}$	240–245	+2.35°	$C_{54}H_{105}NO_{13}.H_2O$	65.21	10.84	65.25	10.67
	DL-Dihydro-sphingosine (*erythro*)	$CH_3(CH_2)_{16}$ [b]	235–240	−3.4°	$C_{48}H_{93}NO_{13}.H_2O$	63.33	10.52	63.68	10.42
		$CH_3(CH_2)_{14}$ [b]	235–240	−3.8°	$C_{46}H_{89}NO_{13}.H_2O$	62.62	10.39	62.67	10.54
		$CH_3(CH_2)_{12}$	230	−6.2°	$C_{44}H_{85}NO_{13}.H_2O$	61.86	10.26	62.33	10.16
		$CH_3(CH_2)_{10}$	220	−4°	$C_{42}H_{81}NO_{13}.H_2O$	61.06	10.12	60.70	10.15
		$CH_3(CH_2)_8$	203–205	−3.2°	$C_{40}H_{77}NO_{13}.H_2O$	60.19	9.97	60.85	9.92
		$CH_3(CH_2)_6$	195–198	−2.1°	$C_{38}H_{73}NO_{13}.H_2O$	59.00	9.81	58.34	9.76
		$CH_3(CH_2)_4$	190–193	−7.8°	$C_{36}H_{69}NO_{13}.H_2O$	58.27	9.64	58.25	9.67
		$CH_3(CH_2)_2$	185–188	−5.3°	$C_{34}H_{65}NO_{13}.H_2O$	57.20	9.46	57.02	9.68
		CH_3 [c]	250	−8.5°	$C_{32}H_{61}NO_{13}.H_2O$	56.03	9.25	55.91	9.19
		Cl_2CH [c]	210	1°	$C_{32}H_{59}NO_{13}Cl_2.H_2O$ [d]	50.92	8.14	51.28	8.40
$CH_3(CH_2)_{12}-CH=CH$	DL-Sphingosine (*erythro*)	Cl_2CH [c]	182	−2.2°	$C_{32}H_{57}NO_{13}Cl_2.H_2O$ [e]	51.06	7.90	51.26	7.22
$CH_3(CH_2)_{14}$	DL-Dihydrosphin-gosine (*threo*)	Cl_2CH	213	−12.3°	$C_{32}H_{59}NO_{13}Cl_2.H_2O$ [f]	50.92	8.14	51.02	8.05
CH_3	DL-Allo-threoninol	$CH_3(CH_2)_{22}$	180–182	+0.3°	$C_{40}H_{77}NO_{13}.H_2O$	60.23	9.98	60.45	10.09
CH_3	DL-threoninol	$CH_3(CH_2)_{22}$	185	+0.1°	$C_{40}H_{77}NO_{13}.H_2O$	60.23	9.98	60.28	9.53
H	Serinol	$CH_3(CH_2)_{22}$	173–175	−1.8°	$C_{39}H_{75}NO_{13}$	61.15	9.87	60.89	10.16

The respective ceramides II were prepared as described earlier: [a] Ref. 6; [b] Ref. 16; [c] Footnote page 54; [d] Calcd. Cl: 9.40; Found: 9.69; [e] Calcd. Cl: 9.42; Found: 9.29; [f] Calcd. Cl: 9.40; Found: 9.46.



Scheme 1



Scheme 2

pared with $-10.8°$[15]) and $-6.4°$ observed with the natural and the synthetic products, respectively[7]). This discrepancy can not be ascribed to the fatty acid, since it was previously shown that in the sphingolipids series the acidic component is an exiguous factor with respect to their physical properties[6,7]). This observation is borne out by a comparison of the lignoceroyl – with the palmitoyl – derivative whose melting points and specific rotations are almost identical (table 2). In view of the comparatively high positive rotation, we are inclined to the assumption that the lactoside reported by these authors contain, at least in part, the α-anomer.

Recently we have described a synthesis of psychosine (1-galactosylsphingosine)* which was based on the protection of the amine function by the dichloroacetyl group. The activated acyl residue could later be removed under very mild alkaline conditions. Following this procedure and employing the dichloroacetyl glycoside (III, $R' = CHCl_2$) we have obtained the corresponding lactosides (IV) of sphingosine, and of *erythro-* and *threo*-dihydrosphingosine. It is noteworthy that the absorption due to the amide C=O stretching bond was found in the dichloroacetyl derivatives to be shifted to 5.9 μ.

Experimental section

Ethyl ***erythro****-α-benzamido-β-hydroxyoctadecanoate (VIa).* Prepared as described previously[8]) for the *threo* methyl ester in 90% yield; m.p. 91–92° (from methanol).

Anal. Calcd. for $C_{27}H_{45}NO_4$: C, 72.47; H 10.13; N, 3.13
Found: C, 72.47; H, 9.94; N, 3.28.

trans*-2-Phenyl-4-carbethoxy-5-pentadecyl-2-oxazoline (VIIa).* Was prepared as described for the *cis*-derivative[8]). The oily product obtained was crystallized from methanol at $-5°$; m.p. 38°.

Anal. Calcd. for $C_{27}H_{43}NO_3$: C, 75.48; H, 10.09
Found: C, 75.04; H, 9.95.

Reduction of the oxazolines VIIb, c with lithium aluminum hydride

The reduction was carried out following essentially the method described for the sphingosine series[8]). After decomposition of the excess of lithium aluminum hydride with ethyl acetate, 10 ml of saturated sodium sulfate solution was added and the mixture was shaken thoroughly to effect complete extraction. The separated inorganic salts were washed with ether and the combined extracts were dried and evaporated. The residue was crystallized twice from hexane, or from hexane-ethyl acetate (3:1).

cis*-2-Phenyl-4-hydroxymethyl-5-methyl-2-oxazoline (VIIIb)*: m.p. 93–94°; yield 30%.

* See footnote on page 54.

Anal. Calcd. for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85; N, 7.33
Found: C, 69.49; H, 6.81; N, 6.94.

trans-2-Phenyl-4-hydroxymethyl-5-methyl-2-oxazoline: m.p. 85–86°; yield 71%.

Anal. Calcd. for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85; N, 7.33
Found: C, 68.94; H, 6.66; N, 7.33.

2-Phenyl-4-hydroxymethyl-2-oxazoline (VIIIc): m.p. 87–88°; yield 68%.

Anal. Calcd. for $C_{10}H_{11}NO_2$: C, 67.78; H, 6.26; N, 7.91
Found: C, 67.70; H, 6.22; N, 8.10.

3-O-*Benzoyl-N-acylamides (II)*. The substituted ceramides were prepared from I in 80–90% yield following the general procedure described in parts VI[6]) and X of this series. The raw products crystallized from methanol, except the butyryl-ceramide and the derivatives of threoninol and serinol for which acetonitrile was used.

threo-N-Dichloroacetyldihydrosphingosine. Removal of the benzoyl group from II, $R' = CHCl_2$ by transesterification in the presence of sodium methylate as described below for the lactosides gave 78% of the amide m.p. 81–82° (from methanol).

Anal. Calcd. for $C_{20}H_{39}NO_3Cl_2$: C, 58.25; H, 9.53; N, 3.43; Cl, 17.20
Found: C, 58.08; H, 9.44; N, 3.23; Cl, 17.00

General procedure for the preparation of ceramide lactosides (III)

The benzoylceramide II (2.5 mM) was dissolved in a mixture of dry benzene (40 ml) and dry nitromethane (40 ml), and moisture was removed by azeotropic distillation of the solvent (30 ml). The solution was allowed to cool to room temperature and, after the addition of 2.5 mM each of heptaacetyl-α-D-lactosyl bromide and mercuric cyanide, stirred with complete exclusion of moisture at 80° for 16–20 hr. The clear filtrate was then shaken several times with a cold saturated solution of hydrogen sulfide until all the salts were removed. Ether was added to facilitate separation of the layers, and the black precipitate was filtered off by suction, if necessary. The combined extracts were washed with a cold 2.5% solution of sodium hydrogen carbonate, then with water to neutral, dried and evaporated. The oily residue was warmed *in vacuo* at 55–60° for 30 min to remove the nitromethane as completely as possible.

The remaining semisolid resulting from the aglucons IIa, b were passed through a silica gel column and eluted with hexane-benzene 3:1. Most of the products were saponified directly, while the heptaacetyl dichloroacetyl derivative was isolated in analytically pure form. For deacylation, the glycosidic ester was dissolved in dry methanol (30 parts), a methanolic solution of sodium (approximately 50 mg) was added, and the mixture was allowed to

stand at room temperature overnight. Neutralization with 2 N acetic acid followed by the addition of ice-water precipitated the lactoside which was filtered and washed thoroughly with distilled water. The dried product was eluted from a silicic acid column by chloroform-methanol (4:1) and was recrystallized either from chloroform-methanol or pyridine acetone. Occasionally, the product (such as the dichloroacetyl derivative) was eluted with a solvent ratio of 9:1. The lactosides were shown to be homogeneous compounds by thin layer chromatograms prepared on Kieselgel G (Merck) and developed with chloroform-methanol-water 75:25:4. The yields amounted to 20–25% except for the dichloroacetyl derivative which was obtained in a yield of 40–45% after crystallization from methanol-acetonitrile.

A high yield was also obtained with the lactosides of *allo*-threoninol, threoninol and serinol, whose heptaacetyl derivatives were eluted from a silicic acid column with chloroform, after removal of impurities with hexane-benzene (1:1), and with benzene. Because of the unfavorable solubility properties of the final products, the usual column technic could not be applied. The three lactosides were then purified by repeated crystallization from methanol or from a mixture of methanol and acetonitrile.

*1-0-(Heptaacetyl-β-**D**-lactosyl)-N-dichloroacetyl-3-0-benzoyl-**DL**-erythro-dihydrosphingosine*: crystallized from hexane and a little benzene; m.p. 60–62°; $[\alpha]_D^{25} + 6.5°$ (C = 1.5 in chloroform).

Anal. Calcd. for $C_{53}H_{77}NO_{21}Cl_2$: C, 56.07; H, 6.8
Found: C, 55.75; H, 6.61.

The lactosides of unsubstituted sphingosine and dihydrosphingosine (IV) were prepared in 60–65% yield by saponification of the corresponding dichloroacetyl derivatives IIIa, b ($R' = Cl_2CH$) following the procedure described earlier.*

*1-0-β-**D**-Lactosyl-**DL**-erythro-sphingosine*: m.p. 225°; $[\alpha]_D^{25} + 0.6°$.

Anal. Calcd. for $C_{30}H_{59}NO_{13}$: C, 56.14; H, 9.27
Found: C, 55.64; H, 9.69.

*1-0-β-**D**-Lactosyl-**DL**-**erythro**-dihydrosphingosine*: m.p. 228–230°; $[\alpha]_D^{25} - 6.3°$.

Anal. Calcd. for $C_{30}H_{61}NO_{13}$: C, 55.95; H, 9.55
Found: C, 55.80; H, 9.36.

*1-0-β-**D**-Lactosyl-**DL**-**threo**-dihydrosphingosine*: m.p. 240°; $[\alpha]_D^{25} - 3°$.

Anal. Calcd. for $C_{30}H_{61}NO_{13}$: C, 55.95; H, 9.55
Found: C, 54.98; H, 9.46.

* See footnote on page 54.

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