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Studies in the Sphingolipids Series. VIII*. Synthesis of *N*-Benzoylsphingine Glucosides, Compounds Related to Cerebrosides

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A description is given for the preparation of some glucosides related to cerebrosides. Optically active and inactive *N*-benzoylsphingine (II), as well as hexadecanol yielded with tetrabenzoyl-α-D-bromoglucose in the nitromethane solution and in the presence of mercuric cyanide the respective glucosides.

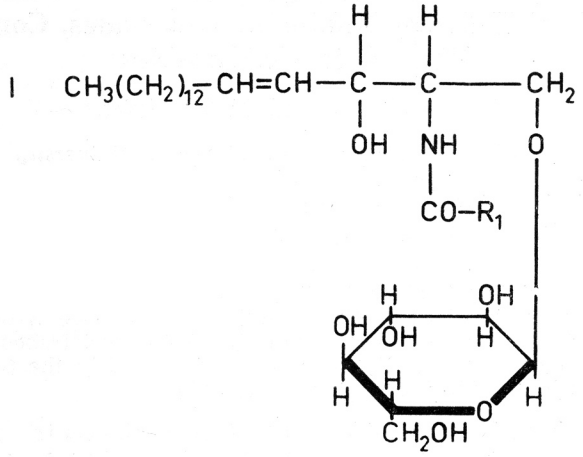
Cerebrosides have been found as constituents of the central nervous system and of other tissues[1]. The structure was deduced on the basis of hydrolysis experiments which yielded sphingosine or dihydrosphingosine, fatty acids and galactose or glucose, the latter being present in certain pathological conditions. Cerebrone (phrenosine), kerasine, nervone and oxynervone were isolated from the nerve tissue, differing from each other in the nature of the fatty acid present (lignoceric, cerebronic, nervonic and oxynervonic). It has also been established recently that sphingosine is D-erythro-2-amino-1,3-dihydroxy-4-trans-octadecene. Pryde and Humphreys[2] found that galactose in cerebrosides contains a hexopyranoside ring. Nakayama[3], Kiss[4] and Carter and Greenwood[5] established that in cerebrone the galactose molecule is linked to the hydroxyl group which is at carbon atom 1 in the sphingosine skeleton. Finaly, Kiss and Jurcsik[6] proved the existence of the α-linkage of galactose in cerebrosides. All these data confirm the structure I for this branch of sphingolipids.

So far as we know the only assay toward synthesis of a cerebroside was reported in 1955. by Kiss[7] at the IInd Congress of Hungarian Chemists. Dihydrocerebrone and dihydrokerasine were partially synthesized from natural dihydrosphingosine. The latter was acylated with tetracosanoic acid chloride and D-2-acetoxytetracosanoic acid chloride and the resulting ceramides treated with tetraacetylbromogalactose in the presence of silver carbonate or mercuric acetate. The β-galactosides thus obtained were converted into the α-isomers, i. e. dihydrocerebrone and dihydrokerasine by means of titanium tetrachloride. Unfortunately, no experimental details were published up to now.

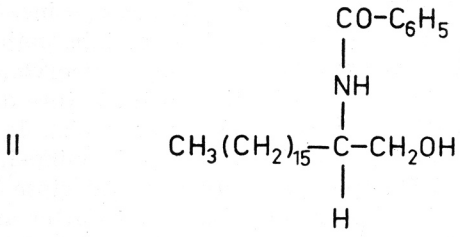
The investigations to be described below concern model experiments. We shall report the synthesis of *N*-benzoylsphingine glucosides. The sphingine (1-hydroxy-2-aminooctadecane) necessary for this purpose was prepared synthetically and resolved into both antipodes according to the known procedure[8].

* Paper VII, M. Prošťenik, *Croat. Chem. Acta* **28** (1956) 287.

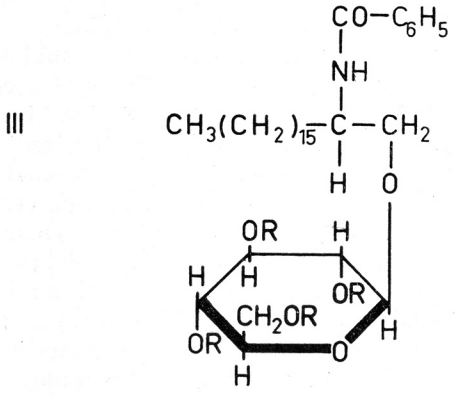
The reaction was carried out with both N-benzoyl-DL-sphingine and N-benzoyl-L-sphingine (II) which were treated with tetrabenzoyl-α-D-bromoglucose (benzobromoglucose) in nitromethane solution and in the presence of mercuric cyanide. This procedure for the preparation of glucosides by means of benzobromoglucose instead of acetobromoglucose was recommended by Helferich



I



II



III

a) $R=C_6H_5CO-$
b) $R=H-$

and Weis[9]. The most useful advantage of benzobromoglucose lies in its relatively high stability. This property was especially valuable in our experiments where the reaction proceeded slowly and at higher temperatures. It should be emphasized that acetobromoglucose was quite unfavourable for this purpose with respect to the yields of the expected glucosides. At any rate, the yields were rather low even when benzobromoglucose was used, the fact being especially evident when the reaction with racemic II was run. This is probably due to the formation of two epimeric glucosides. Starting with racemic II we obtained pentabenzoylglucoside, m. p. 119—121.5° in a very poor yield. The optically active II yielded tetrabenzoyl-1-D-β-glucopyranosyl-L-2-benzamido-1-hydroxyoctadecane (IIIa), m. p. 98—104°, $[\alpha]_D$ — 28,61° (in chloroform). The latter was converted smoothly with sodium methoxide solution into 1-D-β-glucopyranosyl-L-2-benzamido-1-hydroxyoctadecane (IIIb), m. p. 144—145°,$[\alpha]_D$ — 25.69° (in absolute ethanol).

In addition to the above experiments we have found that simple higher aliphatic alcohols such as n-hexadecanol react easier giving satisfactory yields on hitherto undescribed glucosides. Thus, tetrabenzoyl-1-D-β-glucopyranosyl-1-hydroxyhexadecane, m. p. 64—68°, $[\alpha]_D$ + 15.65° (in chloroform) was prepared as well and gave by hydrolysis 1-D-β-glucopyranosyl-1-hydroxyhexadecane, m. p. 137.5°,$[\alpha]_D$ — 16.19° (in chloroform).

These results show obviously that the synthetic formation of higher aliphatic — relatively simple — glucosides is encountered with considerable difficulties which are increasing with the complexity of the higher alcohol used. This fact should be considered with respect to the potential total synthesis of cerebrosides.

EXPERIMENTAL

The melting points are uncorrected.

Tetrabenzoylhexadecyl-β-D-glucoside

A mixture of 2.64 g. of benzobromoglucose, 0.98 g. of hexadecanol, 14 ml. of nitromethane and 1.02 g. of mercuric cyanide was heated for 9 hrs. in a 50 ml. round-bottomed flask at 45—50° with stirring. Nitromethane was then evaporated *in vacuo* and the residue dissolved in benzene. The undissolved mercuric bromide was removed by filtration and the benzene solution chromatographed through a column of 90 g. aluminum oxide (Riedel-de Haën). The first 200 ml. of benzene eluate gave after evaporation of the solvent and recrystallization of the residue from absolute ethanol 1.25 g. *tetrabenzoylhexadecyl-β-D-glucoside,* m. p. 60—64°. The next 200 ml. portion of the benzene eluate gave after evaporation and crystallization from absolute ethanol 0.15 g. of the product, m. p. 64—65.5° (total yield 42.7%). From the following benzene fractions some unreacted hexadecanol was isolated. For analysis the substance was recrystallized from absolute methanol; m. p. 64—68°, $[\alpha]_D^{18}$ + 15.65° (c 2.42, in chloroform).

Anal. 8.090 mg. subst.: 21.73 mg. CO_2, 5.52 mg. H_2O
$C_{50}H_{60}O_{10}$ (820.98) calc'd: C 73.15; H 7.37%
found: C 73.30; H 7.64%

Hexadecyl-β-D-glucoside

To a solution of sodium methoxide, prepared by dissolving of 0.122 g. of sodium in 6 ml. of absolute methanol, was added a solution of tetrabenzoylhexadecyl-β-D-glucoside (0.5 g.) in chloroform (5 ml.). The mixture was allowed to stand for 24 hrs

at —5°. It was then diluted with water (15 ml.), neutralized with *N* hydrochloric acid and evaporated *in vacuo* at 40—50° to dryness. The residue was dissolved in benzene and some undissolved material removed by filtration. The evaporation of the solvent yielded 0.17 g. (68.9%) of a crude, crystalline product, m. p. 125—127°. For analysis the substance was recrystallized twice from petroleum ether and twice from acetonitrile. Colourless crystals, m. p. 137.5° (sint. from 60°); $[\alpha]_D^{24}$ —16.19° (c 1.29, in chloroform).

Anal. 8.380 mg. subst.: 20.11 mg. CO_2, 8.30 mg. H_2O
$C_{22}H_{44}O_6$ (404,57) calc'd: C 65.31; H 10.96%
found: C 65.49; H 11.08%

*Tetrabenzoyl-1-*D*-β-glucopyranosyl-*L*-2-benzamido-1-hydroxyoctadecane* (IIIa)

A mixture of benzobromoglucose (3.22 g., 4.88 mM), *N*-benzoyl-L-sphingine (II) (1.9 g., 4.88 mM), mercuric cyanide (1.23 g., 4.88 mM) and nitromethane (20 ml.) was stirred for 8 hrs. at 80°. The solvent was then evaporated *in vacuo* to dryness and the residue treated with hot ethyl acetate. The turbid solution was filtered, the filtrate evaporated *in vacuo* to dryness and the crystalline residue recrystallized from acetonitrile. An amount of 2.38 g., m. p. 88—89°, was obtained which was recrystallized repeatedly from acetonitrile and benzene to give 0.76 g. of unreacted II, m. p. 112°. The collected benzene and acetonitrile mother liquors were evaporated to dryness, the residue dissolved in benzene and chromatographed on activated alumina (Riedel-de Haën). The ether eluate gave 1.16 g. of a product which was recrystallized from acetonitrile to give 0.8 g. (17%) colourless crystals, m. p. 95—102°. For analysis the substance was recrystallized three times from acetonitrile; m. p. 98—104°, $[\alpha]_D^{20}$ —28.61° (c 1.83, in chloroform).

Anal. 8.700 mg. subst.: 23.27 mg. CO_2, 5.26 mg. H_2O
7.920 mg. subst.: 0.108 ml. N_2 (27.5°, 748 mm)
$C_{59}H_{69}O_{11}N$ (968.15) calc'd: C 73.19; H 7.18; N 1.45%
found: C 72.99; H 6.77; N 1.52%

*Preparation of pentabenzoyl-β-*D*-glucoside from N-benzoyl-*DL*-sphingine*

A mixture of benzobromoglucose (1.32 g., 2 mM), *N*-benzoyl-DL-sphingine (0.78 g., 2 mM), mercuric cyanide (0.51 g., 2 mM) and nitromethane (13 ml.) was stirred for 8 hrs at 70°. The reaction mixture was worked up as described above. An amount of 0.41 g. of unreacted II was obtained. The ether eluates gave 0.63 g. of a crude substance which after several crystallizations from petroleum ether and acetonitrile yielded 0.03 g. (1.6%) of a product melting at 110—115°. Two more crystallizations from acetonitrile gave colourless crystals, m. p. 119—121.5° .

Anal. 6.170 mg. subst.: 16.64 mg. CO_2, 3.91 mg. H_2O
8.475 mg. subst.: 0.113 ml. N_2 (19°, 751 mm)
$C_{59}H_{69}O_{11}N$ (968.15) calc'd: C 73.19; H 7.18; N 1.45%
found: C 73.60; H 7.09; N 1.54%

*1-*D*-β-Glucopyranosyl-*L*-2-benzamido-1-hydroxyoctadecane* (IIIb)

To a solution of sodium methoxide, prepared by dissolving 0.029 g. of sodium in 5 ml. of absolute methanol, a solution of IIIa (0.44 g., 0.454 mM) in chloroform (5 ml.) was added and allowed to stand for 24 hrs at —5°. The reaction mixture was worked up in the same manner as described for the preparation of hexadecylglucoside. The crude substance (0.25 g.), m. p. 143—146°, was recrystallized from acetonitrile. It melted then at 144—145°; $[\alpha]_D^{24}$ —25.69° (c 2.32, in absolute ethanol).

Anal. 9.105 mg. subst.: 22.55 mg. CO_2, 7.84 mg. H_2O
7.955 mg. subst.: 0.177 ml. N_2 (24°, 750 mm)
$C_{31}H_{53}O_7N$ (551.74) calc'd: C 67.48; H 9.68; N 2.54%
found: C 67.59; H 9.64; N 2.53%

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REFERENCES

1. For references covering this field see: H. Thierfelder and E. Klenk, *Die Chemie der Cerebroside und Phosphatide*, Berlin 1930; S. J. Thannhauser and G. Schmidt, *Physiol. Rev.* **26** (1946) 275; W. D. Celmer and H. E. Carter, *Physiol. Rev.* **32** (1952) 167. See also: H. E. Carter and C. G. Humiston, *J. Biol. Chem.* **191** (1951) 727; J. Kiss, G. Fodor, and D. Bánfi, *Chemistry & Industry* 1954, 517; *Helv. Chim. Acta* **37** (1954) 1471; K. Mislow, *J. Am. Chem. Soc.* **74** (1952) 5155; G. Fodor and J. Kiss, *Nature* **171** (1953) 651; H. E. Carter, D. Shapiro, and J. B. Harrison, *J. Am. Chem. Soc.* **75** (1953) 1007, 4705; E. F. Jenny and C. A. Grob, *Helv. Chim. Acta* **36** (1953) 1454; M. Prošťenik, M. Munk-Weinert, and D. E. Sunko, *J. Org. Chem.* **21** (1956) 406.
2. J. Pryde and R. W. Humphreys, *Biochem. J.* **18** (1924) 661.
3. T. Nakayama, *J. Biochem. Japan*, **37** (1950) 309.
4. J. Kiss, Lecture delivered at the Congress for Organic Chemistry, Szeged, September 1952.
5. H. E. Carter and F. L. Greenwood, *J. Biol. Chem.* **199** (1952) 283.
6. J. Kiss and I. Jurcsik, *Acta Chim. Acad. Sci. Hung.* **5** (1955) 477.
7. J. Kiss, *Abstracts of Papers*, IInd Congress og Hungarian Chemists, Budapest, November 1955, p. 39.
8. D. E. Sunko and M. Prošťenik, *J. Org. Chem.* **18** (1953) 1523.
9. B. Helferich and K. Weis, *Chem. Ber.* **89** (1956) 314.

IZVOD

Studije u redu sfingolipoida VIII. Sinteza *N*-benzoil-sfingin glukozida, spojeva srodnih cerebrozidima

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Modificiranom glukozidskom sintezom pripravljeni su iz *N*-benzoil-L-sfingina (II) kao i iz DL-oblika, benzobromglukoze i merkuri-cijanida u nitrometanu, spojevi srodni cerebrozidima. Postupak je iskušan na primjeru pripravljanja znatno jednostavnijeg heksadecil-glukozida. Opisani su ovi spojevi: 1. Tetrabenzoil-heksadecil-β-D-glukozid, t. t. 64—68°, $[\alpha]_D$ + 15.65° (u kloroformu); 2. heksadecil-β-D-glukozid, t. t. 137.5°, $[\alpha]_D$ — 16.19° (u kloroformu); 3. tetrabenzoil-1-D-β-glukopiranozil-L-2-benzamido-1-hidroksi-oktadekan (IIIa), t. t. 98—104°, $[\alpha]_D$ — 28.61° (u kloroformu); 4. benzoilirani glukozid iz N-benzoil-DL-sfingina, t. t. 119—121,5°; 5. 1-D-β-glukopiranozil-L-2-benzamido-1-hidroksi-oktadekan (IIIb), t. t. 144—145°, $[\alpha]_D$ — 25.69° (u aps. etanolu).

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