LXXXVII. THE METHYLATION OF THE CEREBROSIDES OF OX BRAIN.

(PRELIMINARY NOTE.)

BY JOHN PRYDE AND ROBERT WILLIAM HUMPHREYS.

From the Physiology Institute, Welsh National School of Medicine, Cardiff.

(Received April 27th, 1924.)

A SERIES of investigations has been commenced in this laboratory in which it is hoped to apply to substances of biochemical interest, containing a sugar or similar residue, the methylation reaction which in the hands of Irvine [1923] has yielded results of the greatest importance in elucidating the structure of the simple and complex carbohydrates.

The present note deals with some preliminary results which have been obtained in a study of the cerebroside phrenosin and kersin, first isolated and studied in 1874 by Thudichum [1901], and later investigated notably by Thierfelder [1890], Rosenheim [1913], and Levene [1913]. From the results of these and other authors, it is clear that the two cerebroside mentioned are galactosides in which the non-sugar residue consists of a complex formed from the base sphingosine ($C_{17}H_{35}O_2N$), and the fatty acids, phrenosinic acid ($C_{25}H_{50}O_3$) in the case of phrenosin, and lignoceric acid ($C_{24}H_{48}O_2$) in the case of kersin. The constitutional formulae which accord best with their known properties are those of Rosenheim [1916]. So far no attention has been directed to the sugar residue of these compounds *as it occurs in situ* beyond its identification as galactose [Thierfelder, 1890]. Whilst all the naturally occurring glucosides so far examined have been shown [Irvine, 1923] to contain the stable 1 : 4 type of sugar, galactose is remarkable in view of the comparative ease with which it yields four isomeric pentacetates [Hudson, 1915; Hudson and Johnson, 1916] (divided into two pairs of ring isomers), and recently one of us [Pryde, 1923] has brought forward evidence in favour of the view that *normally* galactose does not possess the butylene oxide (1 : 4) internal linkage as hitherto assumed, but an amylene oxide bridge. The nature of the internal linkage of this hexose in naturally occurring galactosides such as the cerebroside is therefore of interest in view of the possibility that it may differ from that of the free sugar, and that opportunities for testing this possibility are limited by the rarity of simple galactosides in nature. It is towards the solution of

this problem that the present investigation is mainly directed. The scheme of work embodies the complete substitution of the hydroxyl groups in the sugar residue by methoxyl, and the subsequent hydrolysis of the methylated complex by acid methyl alcohol. By this means the sugar fragment is obtained as a methylated methyl galactoside—since dry acid methyl alcohol brings about simultaneous hydrolysis and galactoside formation—in which the original position of the internal oxygen bridge is preserved unchanged.

The cerebroside has been isolated from fresh ox brain using the pyridine method of Rosenheim [1914], and have been purified by repeated recrystallisation from alcohol-chloroform mixtures. As a preliminary to the main investigation, and in view of the fact that these compounds contain an unsaturated ethylenic linkage and a secondary nitrogen, it was necessary to discover if they could be submitted to the methylation reaction without degradation, and to establish conditions for this reaction and for the subsequent hydrolysis necessary for the isolation of the methylated fragments, more especially of the sugar residue. For this purpose small quantities of pure phrenosin and kersin, originally prepared by Rosenheim, for which the authors beg to thank Dr J. A. Hewitt of King's College, London, have been subjected to methylation by means of dry silver oxide and methyl iodide.

Kersin and phrenosin undergo methylation unchanged when submitted to the action of the agents mentioned above in a suitable solvent medium, six applications of the process being necessary to ensure complete substitution of the free hydroxyl groups. The initial stages of the methylation are tedious in view of the physical difficulties of solution, and the tendency of the partially methylated cerebroside to separate during extraction as voluminous gels in the presence of such solvents as chloroform. As methylation proceeds these difficulties lessen and the compounds become more and more soluble in methyl iodide, rendering the use of extraneous solvents unnecessary. The results obtained in the case of kersin are in agreement with the work of previous investigators, in that this cerebroside is found to take up five methyl residues to form pentamethylkersin, which was obtained as a white, amorphous, pulverisable compound, melting at about 73°. In the case of phrenosin, however, methylation ceased at a stage intermediate between that required for a pentamethyl- and a hexamethyl-phrenosin, the product resembling pentamethylkersin but having a lower melting point (35–40°). In view of the fact that two forms of this cerebroside have been reported, one containing one molecule of water less than the other, and if this loss of water involved the formation of an internal anhydride [Rosenheim, 1916], such a result might be anticipated if one were dealing with a mixture of the two forms. On the other hand this result is akin to the steric hindrance encountered in the methylation of the amyloses [Pringsheim and Persch, 1921, 1922]. The methylated phrenosin was, like the parent cerebroside, slightly dextro-rotatory, having $[\alpha]_{5461}^{16} + 7.5^\circ$ in methyl alcohol ($c = 0.53\%$). The course of the two methylations is summarised in the following table:

Methylation of kerasin. Wt. used 1.47 g.

No. of methylation	Reagents	Extraneous solvent	Extractive solvent	Methoxyl content (%)	Melting point
1	20 cc. CH_3I 5 g. Ag_2O	30 cc. MeOH	$\text{EtOH} : \text{CHCl}_3$ 1 : 2	6.0	—
2	15 cc. CH_3I 4 g. Ag_2O	10 cc. MeOH	Hot EtOH	8.5	63°
3	7 cc. CH_3I 4 g. Ag_2O	—	Warm EtOH	13.15	45°
4	6 cc. CH_3I 3.5 g. Ag_2O	—	EtOH and a little MeOH	16.1	40–45°
5	6 cc. CH_3I 2 g. Ag_2O	—	EtOH	17.4	50°
6	5 cc. CH_3I 2 g. Ag_2O	—	EtOH	17.68	73°

Pentamethylkerasin requires 17.88 % OMe.

Methylation of phrenosin. Wt. used 1.1 g.

No. of methylation	Reagents	Extraneous solvent	Extractive solvent	Methoxyl content (%)	Melting point
1	7 cc. CH_3I 5 g. Ag_2O	5 cc. MeOH	Hot EtOH	3.6	—
2	7 cc. CH_3I 4 g. Ag_2O	3 cc. MeOH	„	5.8	75–80°
3	6 cc. CH_3I 4 g. Ag_2O	—	„	16.64	35–38°
4	6 cc. CH_3I 3.5 g. Ag_2O	—	„	18.27	35°
5	6 cc. CH_3I 2 g. Ag_2O	—	„	18.6	35°
6	6 cc. CH_3I 2 g. Ag_2O	—	„	18.75	35–40°

Hexamethylphrenosin requires 20.27 % OMe.

It is of interest to note that whilst the melting point of methylated kerasin falls and subsequently rises during methylation, such a rise was not observed in the case of phrenosin. This is in agreement with the suggestion already made that the latter contained an admixture of a second form.

The above preparation of pentamethylkerasin was subjected to hydrolysis by two methods: (1) the action of 2 % hydrochloric acid in dry methyl alcohol in a sealed tube at 100° for 20 hours; (2) boiling under reflux in 7 % sulphuric acid dissolved in dry methyl alcohol for 8 hours. In the latter case the alcoholic solution, after being neutralised with barium carbonate and filtered, deposited on cooling fine, white, feathery crystals, and oily globules which solidified on standing. The former consisted of the methyl ester of the fatty acid residue, and the latter of the sulphate of the base, but these were not further investigated. The methyl alcoholic solution was evaporated, more of the fatty acid ester was deposited, and finally, after removing the last traces of solvent at 100°/10 mm., the product was extracted with ether. The ethereal extract was evaporated, and after treatment in a vacuum at 100° gave a syrupy residue which was fractionally distilled in a high vacuum. A mobile colourless syrup distilled

over at $130^{\circ}/1.0$ mm., and was thus easily separated from the admixture of fatty acid ester which remained in the still. Working on this small test scale it was not possible to obtain more methylated sugar than sufficed for a determination of the refractive index (Abbé-Zeiss) and a small scale determination of the methoxyl content. The results found were n_D^{15} 1.4497 and OMe = 57.5 % and, whilst these are a little divergent from those required for a pure methyl pentamethylgalactoside (n_D^{13} 1.4484; OMe = 62.0 %), they clearly indicate that a separation of the methylated sugar residue is possible by the methods employed. It remains to carry these out on a scale capable of yielding sugar sufficient for its purification and for a full determination of its constitution.

The expense of this research is being defrayed by a grant from the Medical Research Council. One of us (R. W. H.) is indebted to the Department of Scientific and Industrial Research for a scholarship which enables him to take part in this investigation.

REFERENCES.

- Hudson (1915). *J. Amer. Chem. Soc.* **37**, 1591.
Hudson and Johnson (1916). *J. Amer. Chem. Soc.* **38**, 1223.
Irvine (1923). *J. Chem. Soc.* **123**, 898.
Levene (1913). *J. Biol. Chem.* **15**, 359.
Pringsheim and Persch (1921). *Ber. deutsch. chem. Ges.* **54**, 3162.
—— (1922). *Ber. deutsch. chem. Ges.* **55**, 1425.
Pryde (1923). *J. Chem. Soc.* **123**, 1808.
Rosenheim (1913). *Biochem. J.* **7**, 604.
—— (1914). *Biochem. J.* **8**, 110.
—— (1916). *Biochem. J.* **10**, 142.
Thierfelder (1890). *Z. physiol. Chem.* **14**, 209.
Thudichum (1901). *Die chemische Konstitution des Gehirns des Menschen und der Tiere.* Tübingen (Pietzcker).