A New Type of Macrocyclic Lactone from *Torulopsis apicola*

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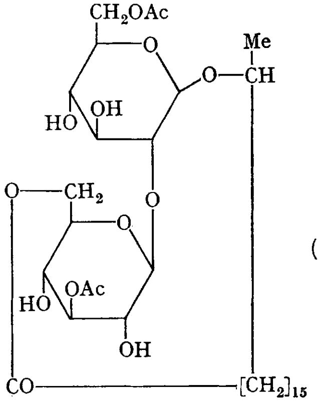
SEVERAL years ago it was discovered that fermentation of octadecane by the yeast *Torulopsis apicola* (formerly *T. magnoliae*) produces a mixture of partially acylated sophorosides of 17-hydroxyoctadecanoic acid.[1] A crystalline component (m.p. 104—105°, *M* 690) isolated subsequently (1962) from the mixture has been found to be a di-*O*-acetylsophoroside in which the carboxyl group is attached to the sugar portion to form a macrocyclic lactone ring. Very recently Jones[2] has suggested structure (I) for a lactone produced by a strain of *Torulopsis* (which had been obtained from this laboratory). Comparison of the lactones in both laboratories indicates that they are identical. However, our studies show instead that the compound is the 4″-lactone of 6′,6″-di-*O*-acetyl-β-sophorosyl-17-hydroxyoctadecanoic acid (II). The main evidence supporting structure (II) is as follows:

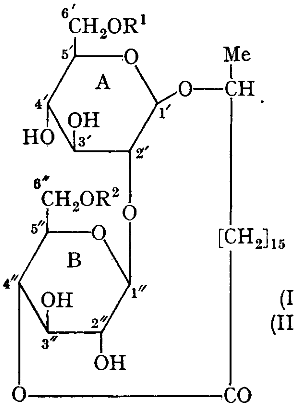
The lactone contains two glycol groups. One of these is readily oxidized by sodium periodate and the oxidation product, on reduction and hydrolysis, yielded glycerol, glycerose, and glucose (approx. 1 : 1 : 1). The second glycol group is unreactive but, together with the first, is cleaved by lead tetra-acetate in pyridine (a reagent known to cleave "resistant glycols"[3]). Reduction and hydrolysis of the fully oxidized product yielded erythritol rather than glucose (as well as glycerol and glycerose). These results indicate that the resistant diol is at the 2,3-position of ring B and the readily oxidized diol at the 3,4-position of ring A. The acyl groups must therefore be attached to C-6′, C-6″, and C-4″. This conclusion was confirmed by the n.m.r. spectrum (in perdeuteroacetone), which shows signals ascribable to H-6″ at δ 4·12 and H-6′ at δ 4·18—4·30, values characteristic for the chemical shift of H-6 in acylated glucosides. The signals of the anomeric protons are at δ 4·44 and 4·60 and a single low-field triplet for the proton of a secondary acyl grouping is observed at δ 4·88. Decoupling experiments confirmed that the third acyl group is at C-4″ since irradiation of H-2′ or H-2″ collapsed one or other of the anomeric proton signals, but did not affect the low-field triplet. Also, spectra of model compounds showed that the high-field H-6 signal is due to H-6″ in ring B with the neighbouring 4″-OH acylated, and the low-field H-6 signal due to H-6′ in ring A where 4′-OH is not acylated.

It now remained to show the point of attachment of the lactone ring. Silicic acid chromatography of the crude lactone had yielded as a minor component a second lactone (III) with only one acetate group, m.p. 88—89°. Acetylation of this lactone gave the same lactone hexa-acetate as obtained from lactone (II); therefore, the lactone ring must be attached to the same point in both lactones. The n.m.r. spectrum of (III) confirmed that only one acetate is present, and the chemical shift of H-6 was found to be δ 4·13 showing that the two acyl groups must be at C-4 and C-6 of ring B. Mild sodium methoxide-catalyzed methanolysis of lactone (II) gives the methyl ester of 6′,6″-di-*O*-acetyl-β-sophorosyl-17-hydroxyoctadecanoic acid suggesting further that the lactone ring is at C-4″.

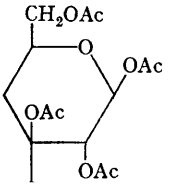
Acetobrominolysis of the fully acetylated lactone, using the conditions of Vis and Fletcher,[4] proceeded readily giving mainly a 17-acetoxyoctadecanoic acid derivative of α-bromosophorose hexa-acetate. However, some cleavage between the glucose rings also occurred giving a fatty-acid ester of α-bromoglucose triacetate. After treatment of the crude product with silver acetate, chromatography on silicic acid gave 4′-*O*-(17-acetoxyoctadecanoyl)-1′,2′,3′,6′-tetra-*O*-acetyl-β-D-glucopyranose (IV). This latter compound was



(I)



(II) R^1=R^2=Ac
(III) R^1=H, R^2=Ac



indistinguishable from the compound synthesized from 1,2,3,6-tetra-*O*-acetyl-β-D-glucopyranose and 17-acetoxyoctadecanoyl chloride, but clearly distinguishable from the isomers with the fatty acid attached at C-6′ or C-3′.

(*Received, May 5th,* 1967; *Com.* 428.)

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