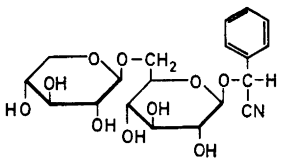
Constitution and Stereochemistry of Lucumin, a Cyanogenic Glycoside from *Lucuma mammosa* Gaertn.

REYNIR EYJÓLFSSON

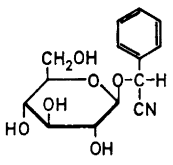
The Royal Danish School of Pharmacy, Chemical Laboratory B, DK-2100 Copenhagen Ø, Denmark

The cyanogenic properties of *Lucuma mammosa* Gaertn. (= *Calocarpum sapota* (Jacq.) Merr.), family Sapotaceae, have been recognized for almost 90 years.[1] However, little attention has been paid to the isolation and characterization of the responsible cyanogenic compound(s). The only publication known to the present author devoted to this problem is that of Bachstez *et al.*,[2] who in 1948 isolated a crystalline, nitrogenous glycoside which they termed lucumin from the seeds of the plant. On the basis of elemental analyses, quoted in the experimental part of the present paper, and qualitative tests they postulated lucumin to be the monohydrate of a diarabinoside of benzaldehyde cyanohydrin. Crystalline acetyl and benzoyl derivatives, the latter seemingly incompletely acylated, were prepared. The authors, after a naive fashion not required by the analytical data, regarded these compounds as hexaacyl derivatives of the glycoside hydrate.

Reinvestigation of this topic has now resulted in the isolation of a cyanogenic glycoside that appears from its melting point and the properties of its acetyl derivative to be identical with the lucumin isolated by Bachstez *et al.* Complete acid-catalyzed hydrolysis of lucumin (I) resulted in the liberation of D-xylose, D-glucose, benzaldehyde, and HCN. Partial acid-catalyzed hydrolysis furnished, *inter alia*, prunasin (II), a cyanogenic glucoside of known constitution and stereochemistry,[3] unambiguously proving the (*R*) configuration of the chiral centre in the aglycone of lucumin as well as the β-glycosidic linkage between the glucose and aglycone moieties. Exhaustive methylation of lucumin followed by hydrolysis afforded 2,3,4-tri-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose, supporting the pyranose forms of both sugar units and the attachment of the xylose to the 6-position of the glucose residue.



I



II

The two previously known 6-glycopyranosides of prunasin are vicianin and amygdalin.[3] Their molecular rotations in H_2O, −89 (calc. as monohydrate) and −176, respectively (*cf.* Ref. 4), differ from the rotations [5] of the corresponding methyl glycosides, the α-L-arabinopyranoside (+28) and β-D-glucopyranoside (−66), by increments (−117 and −110) that are nearly if not quite identical and of the same order of magnitude as the molecular rotation of prunasin (−82), in accord with Klyne's rule.[6] Comparison of the molecular rotations of lucumin, −224, and methyl β-D-xylopyranoside,[5] −108, which differ by the same increment (−116), establishes that lucumin is a β-xyloside and confirms the rest of its structure. The equilibrium molecular rotations of the 6-*O*-glycopyranosyl-D-glucoses, vicianose [7] (+124), gentiobiose [5] (+35), and primverose [8] (−10), whose mutarotation presumably leads to mixtures of equivalent composition, form the same pattern in that the rotation of the β-D-xyloside is 45 ± 3 less than that of the β-D-glucoside and 135 ± 1 less than that of the α-L-arabinoside. The molecular rotation of the α-xyloside corresponding to lucumin would be expected from data on methyl α-D-xylopyranoside [5] and 6-*O*-α-D-xylopyranosyl-D-glucose [8] to be near +150.

In summary, the constitution of lucumin can be expressed in the systematic name 2(*R*)-[6-*O*-(β-D-xylopyranosyl)-β-D-glucopyranosyloxy]-2-phenylacetonitrile. The disaccharide residue in lucumin, primverose, is a sugar rather frequently encountered in plant glycosides (see, *e.g.*, Ref. 9). By contrast, xylose has hitherto not been detected as a part of any other cyanogenic glycoside.[3]

Experimental. ^{1}H NMR and IR spectra were recorded on JEOL JNM-C-60HL and Perkin-Elmer Model 457 instruments, respectively. Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter. Silica gel (Merck, 0.05–0.20 mm) used for preparative

work contained 10 % H_2O. Authentic reference compounds were prepared by conventional procedures. The plant material, seeds of *Lucuma mammosa* Gaertn., was obtained from Mexico with the kind help of Dr. O. Olsen, The Royal Botanical Garden, Copenhagen. The specimen was transported by air to Copenhagen and worked up immediately when received. An authentic sample of the seeds is stored in this laboratory.

Isolation of lucumin. The shells were carefully removed from the seeds and the kernels (230 g) were immediately immersed in boiling absolute EtOH (1.5 l) for 1 h. The supernatant was decanted and evaporated at reduced pressure and its residue mixed with the crushed kernels. The mixture was extracted continuously with petroleum ether in a Soxhlet for 24 h, whereupon the material was finely pulverized and extracted with petroleum ether for a further 24 h. The dried powder was then extracted with MeOH in a Soxhlet for 24 h. Evaporation of the solvent at reduced pressure yielded a dark-brown solid (32.8 g), which was treated with H_2O (500 ml) and centrifuged. The supernatant was clarified by filtration (diatomaceous earth) and diluted with H_2O to approx. 1 l. The solution was extracted continuously with appropriate portions of EtOAc in a liquid-liquid extractor until the last organic extracts gave practically no sign of glycosidic components (silica gel-TLC, naphthoresorcinol-H_2SO_4 reagent).

The pooled EtOAc extracts were concentrated to dryness at reduced pressure, affording a yellow solid (6.3 g). Repeated column chromatography of this material on silica gel (solvent system EtOAc-MeOH-H_2O, 7.9:1.1:1.0, v/v) furnished lucumin monohydrate, crystallized from Bz-MeOH, yield 1.51 g (0.65 % of the fresh kernels), m.p. 184–185° (corr.), (Ref. 2, m.p. 183–184°), $[\alpha]_D^{20}$ −50.5 (*c* 0.4, H_2O). (Found: C 51.21; H 6.19; N 3.15. Calc. for $C_{15}H_{25}O_{10}N.H_2O$: C 51.16; H 6.11; N 3.14. Ref. 2 reports C 51.54, 51.71; H 6.42; 6.55; N 3.98; 4.05). The IR spectrum (KBr disc) revealed a very weak peak at 2250 cm^{-1} (ν_{CN}).

Detection of xylose and glucose as hydrolysis products of lucumin. The glycoside (10.3 mg) was refluxed in 4 N HCl (1 ml) for 30 min. TLC analysis (2 solvent systems) of the cooled, neutralized (Amberlite IR 45, OH^-) solution indicated the presence of xylose and glucose, employing authentic reference compounds.

Detection of benzaldehyde and HCN as hydrolysis products of lucumin. Lucumin (9.0 mg) was treated with 4 N HCl (2 ml) and distilled (distillate vol. *ca.* 1 ml) in a semi-micro still into a receiver containing 2,4-dinitrophenylhydrazine reagent (0.2 % in 4 N HCl) (5 ml). The receiver was fitted airtight to the condenser outlet and was equipped with a small-bore side tube. Test paper strips impregnated with sodium picrate [10] or tetrabase-copper ethyl acetoacetate [11] inserted in this tube indicated the evolution of HCN. An orange precipitate appeared in the 2,4-DNPH reagent, which was shaken with Et_2O. TLC analysis of the Et_2O phase (2 solvent systems) indicated the presence of benzaldehyde 2,4-dinitrophenylhydrazone, using an authentic reference sample.

Partial hydrolysis of lucumin. Lucumin (100 mg) was refluxed in 0.4 N HCl (10 ml) for 30 min. The mixture was evaporated to dryness at reduced pressure (12 mm, 30°) and placed overnight over solid NaOH in vacuum (12 mm) at room temp. TLC analysis of the residue (5 solvent systems) indicated the presence of prunasin, which was isolated by chromatography on silica gel (30 g, solvent system EtOAc-MeOH-H_2O, 9.0:0.5:0.5, v/v). Removal of the solvent afforded a colourless residue (10.6 mg) that on crystallization from Bz-MeOH yielded a compound (5.2 mg) whose IR spectrum (KBr pellet) was superimposable on that of authentic prunasin. $[\alpha]_D^{20}$ −58.1 (*c* 0.3, EtOH). The mother liquor furnished another crop of prunasin (3.6 mg), $[\alpha]_D^{20}$ −59.1 (*c* 0.2, EtOH). (A standard sample had $[\alpha]_D^{20}$ −59.3 (*c* 0.5, EtOH) and $[\alpha]_D^{20}$ −27.9 (*c* 0.4, H_2O)).

Complete methylation of lucumin and identification of its hydrolysis products. Methylation of lucumin (300 mg), essentially after Kuhn *et al.*,[12] followed by chromatographic purification of the product on silica gel (30 g, solvent system Bz-EtOAc, 6:4, v/v) afforded hexamethyllucumin (185 mg) as a colourless syrup. No IR absorption in the 3200–3600 cm^{-1} region. This material (150 mg) was suspended in 2 N HCl (15 ml) and refluxed under vigorous stirring for 2 h. The solution was neutralized (Amberlite IR 45, OH^-) and evaporated to a thick syrup (137 mg). Chromatography of this syrup on silica gel (30 g, solvent system EtOAc at first and then with increasing concentrations of MeOH (up to 4 %, v/v) in EtOAc) gave 2,3,4-tri-*O*-methyl-D-xylose (crystalline), $[\alpha]_D^{20}$ +41.8 (*c* 0.5, MeOH), and 2,3,4-tri-*O*-methyl-D-glucose (syrup), $[\alpha]_D^{20}$ +65.0 (*c* 0.45, MeOH), in almost quantitative yields. The IR spectra of these derivatives were identical with those of the pertinent reference compounds. (Authentic 2,3,4-tri-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose had $[\alpha]_D^{20}$ +42.5 (*c* 0.5, MeOH) and $[\alpha]_D^{20}$ +75.8 (*c* 0.35, MeOH), respectively).

Hexaacetyllucumin. This compound was

obtained by acetylation of lucumin (100 mg) utilizing the pyridine-Ac_2O technique. Crystallized from H_2O-EtOH, yield 134 mg, m.p. 138–139° (corr.), (Ref. 2, m.p. 137–138°). (Found: C 55.00; H 5.56; N 2.09. Calc. for $C_{31}H_{37}O_{16}N$: C 54.77; H 5.39; N 2.06. Ref. 2 reports C 54.68, 54.91; H 5.32, 5.5.) ^1H NMR spectrum (5 % in $CDCl_3$), δ (relative to TMS, internal): 7.46, sharp s, 5 H (aromatic protons); 5.54, sharp s, 1 H (the methine proton of the aglycone); 5.4–3.0, many complex signals, 13 H (sugar protons); 2.15–1.90 (sharp peaks at 2.10, 2.05, 2.00 and 1.98 are readily discernible), 18 H (acetyl protons).

Acknowledgements. The author records his sincere thanks for an institutional postdoctoral scholarship. Elemental analyses were carried out by The Microanalytical Laboratory, University of Copenhagen. Dr. O. Olsen, The Royal Botanical Garden, Copenhagen, is thanked for most valuable help in obtaining the plant material, as well as for botanical identification. Thanks are also due to Professor, Dr. Chr. Pedersen, The Technical University of Denmark, for his gift of β-glucosan, used as starting material in the preparation of 2,3,4-tri-*O*-methyl-D-glucose. The kind help of Professor, Dr. M. Ettlinger, Chemical Laboratory II, University of Copenhagen, concerning the preparation of the manuscript is gratefully acknowledged.

1. Jorissen, A. *Bull. Acad. Sci. Belg.* **53** (1884) 256.
2. Bachstez, M., Santisteban Prieto, E. and Canales Gaja, A. M. *Ciencia (Mexico City)* **9** (1948) 200.
3. Eyjólfsson, R. *Fortschr. Chem. org. Naturstoffe* **28** (1970). *In press.*
4. Hudson, C. S. *J. Am. Chem. Soc.* **46** (1924) 483.
5. Bates, F. J. and associates. *Polarimetry, Saccharimetry and the Sugars, U. S. Nat. Bur. Stand. Circ.* C440, Govt. Printing Office, Washington, D. C. 1942.
6. Klyne, W. *Biochem. J.* **47** (1950) xli.
7. Bertrand, G. and Weisweiller, G. *Compt. Rend.* **150** (1910) 180; *Bull. Soc. Chim. France* **9** (1911) 38.
8. Zemplén, G. and Bognár, R. *Ber.* **72** (1939) 1160.
9. Bailey, R. W. *Oligosaccharides*, Pergamon, Oxford 1965, p. 87.
10. Mirande, M. *Compt. Rend.* **149** (1909) 140.
11. Tantisewie, B., Ruijgrok, H. W. L. and Hegnauer, R. *Pharm. Weekblad* **104** (1969) 1341.
12. Kuhn, R., Egge, H., Brossmer, R., Gauhe, A., Klesse, P., Lochinger, W., Röhm, E., Trischmann, H. and Tschampel, D. *Angew. Chem.* **72** (1960) 805.

Received March 4, 1971.

Studies on Orchidaceae Alkaloids

XXVI.* A New Glycosidic Alkaloid from *Malaxis grandifolia* Schltr.

BJÖRN LINDSTRÖM, BJÖRN LÜNING and KIRSTI SIIRALA-HANSÉN

Department of Organic Chemistry, University of Stockholm, Sandåsgatan 2, S-113 27 Stockholm, Sweden

Most species belonging to the subfamily Liparidinae in Orchidaceae produce large amounts of alkaloid glycosides. The aglucones of these are aminoesters of 3,5-dialkyl substituted *p*-hydroxybenzoic acids.[2,3,4]

From the species *Malaxis grandifolia* Schltr. a new alkaloid (I) has been isolated for which we propose the name grandifoline (Fig. 1). The amorphous alkaloid was

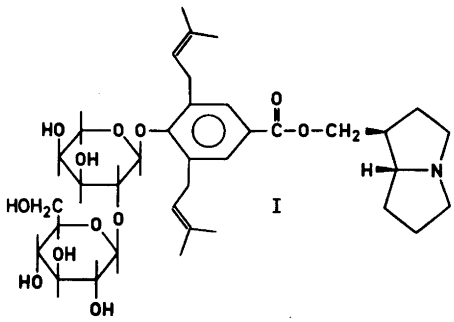


Fig. 1. Grandifoline (I).

* For paper XXV of this series, see Ref. 1.