Simmondsin, an Unusual 2-Cyanomethylenecyclohexyl Glucoside from *Simmondsia californica*

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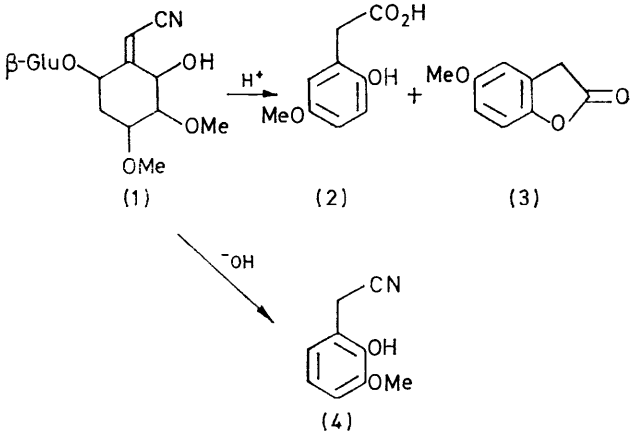
Simmondsin, a monoglucoside extracted from seeds of the jojoba plant (*Simmondsia californica*), has been shown to be 2-(cyanomethylene)-3-hydroxy-4,5-dimethoxycyclohexyl β-D-glucoside (1). Analysis of the n.m.r. spectra of this compound and its penta-acetate permits assignment of the stereochemistry as well as establishing the point of attachment of the glucose.

THE jojoba plant, *Simmondsia californica* (chinensis), is a shrub which grows wild in the south-western United States and north-western Mexico. Its seeds furnish *ca.* 50% of a liquid wax comprised mainly of C_{40}, C_{42}, and C_{44} wax esters [1] as well as a meal containing 32% protein after removal of oil.[2] Although much attention has been given to use of the oil as a potential replacement for spermaceti, relatively little work has been carried out on the use of the residual meal. Preliminary investigations in this laboratory[3] indicate that incorporation of jojoba meal into the diet of weanling rats causes extreme weight loss, and that this reflects failure of the animals to consume food. It was found also that extraction of ground jojoba seed in succession with heptane, benzene, ethyl acetate, and methanol concentrated the active substance or substances in the ethyl acetate fraction. Chromatography of this mixture on silica gel then yielded a component which was obtained crystalline from acetone–methanol and which exhibited activity in the inhibition of feeding, although the acute oral toxicity (LD_{50} > 4 g kg^{-1}) was extremely low. We have termed this compound simmondsin and have assigned it structure (1).

Simmondsin bears a glucose unit which was removed hydrolytically by incubation with β-glucosidase. This established the β-linkage as well as confirming the identity of the sugar unit, which was also compared with authentic glucose by t.l.c. Attempts to isolate the aglucone from the hydrolysate yielded only mixtures of extensively degraded material.

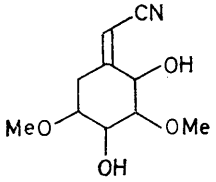


Simmondsin formed a penta-acetate, the n.m.r. spectrum of which clearly showed five acetyl resonances. The observed mass (585·201) of the mass spectral parent ion was consistent with the empirical formula $C_{26}H_{35}NO_{14}$, corresponding to the formula $C_{16}H_{25}NO_9$ for simmondsin itself. The i.r. spectrum of simmondsin revealed bands at 2218 and 1640 cm^{-1}, which are to be expected for an αβ-unsaturated nitrile.[4] The presence of this system is also in agreement with the observed u.v. maximum at 217 nm.[5] These functionalities taken

[1] T. K. Miwa, *J. Amer. Oil Chemists' Soc.*, 1971, **48**, 259.
[2] F. B. Wells, *Cereal Chem.*, 1955, **32**, 157.
[3] A. N. Booth, C. A. Elliger, and A. C. Waiss, unpublished results.
[4] L. J. Bellamy, ' The Infrared Spectra of Complex Molecules,' Wiley, New York, 1954, pp. 263—264.
[5] A. I. Scott, ' Interpretation of the Ultraviolet Spectra of Natural Products,' MacMillan, New York, 1964, pp. 84—85.

together with the molecular formula indicate that the aglucone is monocyclic.

Treatment of simmondsin with acid gave 2-hydroxy-5-methoxyphenylacetic acid (2) and the corresponding lactone (3). The latter has been described [6] and could also be prepared from (2) by treatment with trifluoroacetic anhydride. The acid (2) was also converted into the known 2,5-dimethoxyphenylacetic acid [7] with dimethyl sulphate in refluxing acetone containing potassium carbonate. Basic degradation of simmondsin yielded 2-hydroxy-3-methoxyphenylacetonitrile (4), which was hydrolysed to the acid [8] and subsequently converted into its lactone.[9] The physical properties of these substances are in agreement with reported data. The substitution patterns of the acid (2) and the nitrile (4) limit the structural arrangement of the aglucone to three possibilities (5)—(7).



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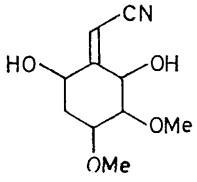

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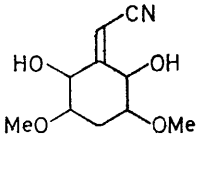


(7)

100 MHz N.m.r. spectra (δ values; J in Hz) of simmondsin and its penta-acetate

Simmondsin (in CD_3OD)	Penta-acetate (in $CDCl_3$)
Ring protons of aglucone	
H-1 5·78 (d, $J_{1,2}$ 2)	5·46
H-2 4·80 (dd, $J_{2,3}$ 9, $J_{1,2}$ 2)	6·04
H-3 3·22 (dd, $J_{2,3}$ 9, $J_{3,4}$ 3)	3·23
H-4 3·98 (complex)	3·84
H-5 1·73 (dt, $J_{5,6}$ 15, $J_{4,5}$ and $J_{5,7}$ 4)	1·62
H-6 2·58 (dt, $J_{5,6}$ 15, $J_{4,6}$ and $J_{6,7}$ 4)	2·44
H-7 4·96 (t, $J_{6,7}$ and $J_{5,7}$ 4)	4·80
Remainder of structures	
OMe 3·53 and 3·56 (s)	3·36 and 3·44
OH 5·4br (s)	
OAc	2·02, 2·04, 2·06, 2·10, and 2·16 (s)
H-8 4·48 (d, $J_{8,9}$ 8)	4·72
H-9—H-14 *ca.* 3·3—3·8 (complex)	H-13 and H-14 4·04 and 4·26 [both dd, $J_{13,14}$ 12, $J_{12,13}$ and $J_{12,14}$ 3 (lower field m) and 4 (higher field m)]
	H-12 3·68 (complex)
	H-9,-10,-11 4·9—5·3 (complex)

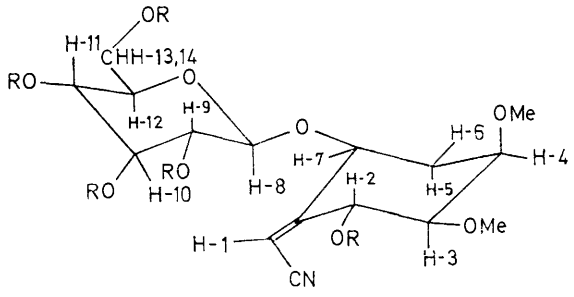
The n.m.r. spectra (Table) of simmondsin and its penta-acetate furnish sufficient information to distinguish between these possibilities as well as to establish the relative configuration of the substituents and the point of attachment of the glucose. The signals associated with the ring protons of the aglucone are distinctly separated from those of the remainder of the molecule, and the respective coupling constants were unambiguously established by spin-decoupling techniques.

[6] Z. Zbiral, E. L. Menard, and J. M. Müller, *Helv. Chim. Acta*, 1965, **48**, 404.

[7] J. M. Gulland and C. J. Virden, *J. Chem. Soc.*, 1928, 1478.

[8] W. Ried and H. G. Gebardtsbauer, *Chem. Ber.*, 1956, **89**, 1933.

Formula (8) expresses the results. The 2 Hz doublet occurring at δ 5·78 assigned to the olefinic proton H-1 of the exocyclic double bond is coupled through the extended system to the axial proton H-2. The angle



R = H or Ac

(8)

between the C–H-2 bond and the plane of H-1 and the C=C bond (*ca.* 90°) is consistent with the observed coupling; the orientation of H-7 nearly within the plane of the double bond would not be expected to give rise to coupling.[10] We have assigned the transoid form shown on the basis of the coupling magnitude. A diaxial relationship exists between H-2 and H-3 ($J_{2,3}$ 9 Hz) thus establishing the respective hydroxy- and methoxy-groups as equatorial. Additionally, the large shift in the resonance of H-2 (δ 4·80 to 6·04) observed on acetylation indicates that the corresponding hydroxy-group is unsubstituted in simmondsin itself. The chemical shifts associated with the signal of H-2 are appropriate for allylic alcohol and allylic acetate. The proton H-4 is coupled to H-3, H-5, and H-6, giving rise to the observed complex signal. The values ($J_{3,4}$ 3, $J_{4,5}$ and $J_{4,6}$ 4 Hz) of coupling constants indicate that H-4 is equatorial. The chemical shift (δ 3·98) of this proton strongly indicates attachment of an oxygen function to the same carbon atom and effectively rules out structure (7) for the aglucone.

Protons H-5 and H-6, attached to unsubstituted carbon atoms, give rise to the ABX_2 multiplet of which the two triplet pairs at δ 1·73 and 2·58 are the higher field component. Irradiation at the frequency assigned to H-4 caused the upfield pattern to simplify into two pairs of doublets (this was also true for irradiation at a frequency appropriate to H-7). Only structure (6) is in agreement with this result. The signal (δ 4·96) associated with H-7 appears as a triplet, which resolves itself into a doublet on irradiation at either of the frequencies corresponding to H-5 and H-6. An equatorial configuration of H-7 is indicated by the observed coupling constants (4 Hz). Attachment of the sugar to the oxygen atom at this position may be inferred from the previous assignment of the free hydroxy-group (*cf.* the relative shifts of H-2 and H-7 in the Table).

In the glucose portion of the simmondsin molecule

[9] W. Moisimann and J. Tambor, *Ber.*, 1916, **49**, 1258.

[10] L. M. Jackman and S. Sternhell, 'Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry,' 2nd edn., Pergamon, New York, 1969, p. 316.

a value of 8 Hz (diaxial) observed for $J_{8.9}$ corroborates the enzymic result in assigning a β-linkage at the anomeric centre.

A number of unusual features characterise the simmondsin molecule. It is difficult to rationalise the preferred axial conformation of the glucose-bearing oxygen atom attached to the cyclohexane ring, although the axial and equatorial methoxy-groups balance each other energetically. Stabilisation of this conformation may be achieved to some extent, however, through association of the equatorial hydroxy-group with the cyano-group on the double bond, which are in close proximity in this conformation. In this respect the results of acidic hydrolysis, in which this equatorial hydroxy-group is one of the groups eliminated during aromatisation, are interesting. A reasonable explanation might lie in the preliminary addition of OH to the nitrile function, forming a cyclic imino-ester which could then undergo ring opening *via* alkyl–oxygen fission, either as such or after conversion into the corresponding lactone. The lability of simmondsin towards base also does not lend itself to ready explanation. Even under very mild conditions (pH *ca.* 9—10) in aqueous base conversion into the nitrile (4) occurs. Seemingly much stronger alkali would be necessary even if such groups as methoxy or β-glucosyl were commonly subject to β-elimination. Here, although both leaving groups are axially oriented, there is no particular activation of β-elimination. Moreover, it was observed that complete etherification of the free hydroxy-groups of the molecule resulted in a material stable towards base; a free hydroxy-group or groups, either attached to the sugar or to the cyclohexane ring, is essential to the elimination.

The biogenesis of simmondsin presents a problem which is probably soluble only through a study of labelling patterns in the plant. An unsaturated nitrile of structure (1) could presumably arise through dehydration of the corresponding cyanohydrin or *via* a condensation onto the cyclohexane system to furnish a short side chain convertible into the nitrile. Dehydroquinic acid suggests itself as a possible precursor.

EXPERIMENTAL

M.p.s were measured on a Thomas–Hoover capillary apparatus and are corrected. I.r. spectra were recorded on a Perkin-Elmer 257 instrument. U.v. spectra were recorded on a Beckman DK-2 spectrophotometer. N.m.r. spectra were measured on a Varian A-60 instrument or on an HA-100 spectrometer. Spin-decoupled spectra were obtained on the latter machine in the frequency mode. Mass spectra were obtained on a C.E.C. 21-110 system at high resolution. Elemental analyses were performed in this laboratory.

Isolation of Simmondsin.—Jojoba seed (250 g) was ground with heptane (250 ml) to a particle size below 1 mm in a Sorvall Omnimixer. The finely divided solid was filtered off and extracted (Soxhlet) with heptane for 4 h; the extract was combined with the initial filtrate to give, after removal of solvent, jojoba oil (123 g). Soxhlet extraction of the remaining meal in succession with the following solvents provided additional fractions: benzene, 24 h (6·48 g); ethyl acetate, 136 h (14·61 g); methanol, 64 h (17·34 g).

The ethyl acetate-extracted material (10 g) was applied in methanol (20 ml) to a column (50 × 1000 mm) prepared with silica gel (900 g) (Mallinckrodt SilicAR CC-7, 200—325 mesh) in chloroform. Elution was carried out at 300 ml h^{-1} in a linear gradient from 100% chloroform to 20% methanol–chloroform (10 l) followed by an additional 5 l of 20% methanol–chloroform. The fraction eluted between 8·4 and 10·2 l was evaporated to give a viscous oil (4·6 g), which was taken up in methanol–acetone (1 : 1; 50 ml). Water (5 ml) was added, and the solution was allowed to evaporate at room temperature to give large crystals of *simmondsin* [2-(*cyanomethylene*)-3-*hydroxy*-4,5-*dimethoxycyclohexyl* β-D-*glucoside*] (3·42 g), melting over the range 95—100° and containing *ca.* 1—1·5 mol equiv. of water (by n.m.r.). A sample dried for 16 h at 80° *in vacuo* (Found: C, 51·1; H, 6·65; N, 3·7. $C_{16}H_{25}NO_9$ requires C, 51·2; H, 6·7; N, 3·75%) had $\lambda_{max.}$ 217 nm (log ε 4·04); $\nu_{max.}$ (KBr) 3400br, 2218, and 1640 cm^{-1}. The n.m.r. spectrum (Table) is discussed in the text.

Simmondsin Penta-acetate.—Simmondsin (0·50 g), potassium acetate (0·50 g), and acetic anhydride (5 ml), were warmed on a steam-bath with occasional shaking for 4 h. Most of the remaining acetic anhydride and acetic acid was then removed under reduced pressure, and the residue was ground with chloroform (25 ml). Filtration, and evaporation of the filtrate gave a solid which was crystallised from ethyl acetate–heptane (1 : 1; 15 ml) to yield *simmondsin penta-acetate* (0·35 g), m.p. 165—166° (Found: C, 53·5; H, 6·05; N, 2·4%; M^+, 585·201. $C_{26}H_{35}NO_{14}$ requires C, 53·35; H, 6·0; N, 2·4%; M^+, 585·206); $\nu_{max.}$ ($CHCl_3$) 2216, 1760, and 1640 cm^{-1}. The n.m.r. spectrum (Table) is discussed in the text.

Enzymic Hydrolysis of Simmondsin.—Simmondsin (0·50 g) was dissolved in acetate buffer solution (pH 5; 0·1M; 10 ml) and a solution of β-glucosidase (β-D-glucoside glucohydrolase, E.C. No. 3.2.1.21) (0·10 g) in buffer (5 ml) was added. Xylene (1 ml) was added to form a layer over the aqueous mixture, and the loosely stoppered container was incubated in a water-bath at 37—40° for 110 h. The hydrolysis was followed by t.l.c. on silica gel plates (Merck Cat. No. 68 10-010-1), with propan-1-ol–ethyl acetate–water (7 : 2 : 1) as eluant. Glucose formation was indicated by appearance of a spot of R_F *ca.* 0·21. Attempts to isolate the aglucone from the hydrolysate by continuous extraction yielded only ill-defined mixtures of aromatic materials.

Treatment of Simmondsin with Base.—Simmondsin (2·13 g), water (20 ml), and sodium hydroxide (0·20 g) were warmed together briefly to complete solution; the flask was purged with nitrogen and set aside for 16 h at room temperature. Hydrochloric acid (conc.) was added (to pH 2) and the mixture was extracted with ether (2 × 20 ml); the extract was dried and evaporated to give 2-*hydroxy*-3-*methoxyphenylacetonitrile* (0·73 g), m.p. 101—103° [from ethanol–water (1 : 1)] (Found: C, 66·4; H, 5·5; N, 8·6. $C_9H_9NO_2$ requires C, 66·25; H, 5·55; N, 8·6%), $\lambda_{max.}$ (95% EtOH) 205infl and 278 nm (log ε 3·77 and 3·45), $\lambda_{max.}$ (95% EtOH, basic) 247 and 294 nm (log ε 3·91 and 3·65); $\nu_{max.}$ ($CHCl_3$) 3550 and 2260 cm^{-1}; δ ($CDCl_3$) 3·72 (2H, s), 3·87 (3H, s), 5·96 (1H, s), and 6·75—7·0 (3H, m).

The nitrile (0·60 g) was hydrolysed in aqueous sodium

hydroxide (15%; 4 h, reflux). Acidification and extraction with ether (2 × 25 ml) gave 2-hydroxy-3-methoxyphenylacetic acid (0·50 g), m.p. 123—125° [from heptane–ethyl acetate (1 : 1)] (lit.,[8] 124°), $\nu_{max.}$ ($CHCl_3$) 3550 and 1735 cm^{-1}; δ ($CDCl_3$) 3·74 (3H, s), 3·83 (2H, s), and 6·84 (3H, s).

Treatment of this acid (0·20 g) with trifluoroacetic anhydride (1 ml; room temp.; 30 min) gave the corresponding lactone, m.p. 77—79° (from heptane) (lit.,[9] 80°), $\nu_{max.}$ ($CHCl_3$) 1815 cm^{-1}; δ ($CDCl_3$) 3·72 (2H, s), 3·92 (3H, s), and 6·73—7·26 (3H, m).

Treatment of Simmondsin with Acid.—Simmondsin (4·40 g) dissolved in N-hydrochloric acid (50 ml) was refluxed for 1·5 h. After most volatile material had been removed under reduced pressure, the mixture was triturated with ethyl acetate (4 × 25 ml). Evaporation gave an oil (2·19 g) which was applied in ethyl acetate (2·5 ml) to a column (25 × 1000 mm) prepared with 190 g of silica gel (Mallinckrodt SilicAR CC-7, special) in chloroform. Elution was carried out at 60 ml h^{-1} in a linear gradient from 100% chloroform to 20% methanol–chloroform (2 l). Three major fractions were obtained, (i) between 370 and 580 ml (0·16 g), (ii) between 860 and 940 ml (0·33 g), and (iii) between 1040 and 1160 ml (1·31 g). Fraction (iii) was dissolved in ethyl acetate (40 ml) and extracted with 5% sodium hydrogen carbonate solution (3 × 30 ml). Acidification of the aqueous extract followed by extraction with ethyl acetate (3 × 20 ml) afforded *2-hydroxy-5-methoxyphenylacetic acid* (2) (0·40 g), m.p. 131—133° (from toluene) (Found: C, 59·6; H, 5·55. $C_9H_{10}O_4$ requires C, 59·35; H, 5·55%), $\nu_{max.}$ (KBr) 3400br and 1725 cm^{-1}; δ[$(CD_3)_2CO$] 3·61 (2H, s), 3·69 (3H, s), 6·5—6·9 (3H, m), and 8·3br (2H, s).

The acid (25 mg) was treated with trifluoroacetic anhydride (0·2 ml; room temp.; 30 min). Evaporation of the volatile material and crystallisation from heptane gave the lactone (3), m.p. 95—98° (lit.,[6] 95°), $\nu_{max.}$ ($CHCl_3$) 1810 cm^{-1}; δ ($CDCl_3$) 3·70 (2H, s), 3·78 (3H, s), and 6·7—7·1 (3H, m).

Treatment of the acid (0·10 g) with dimethyl sulphate (0·5 ml) and potassium carbonate (0·6 g) in acetone (25 ml; reflux; 19 h), followed by filtration and evaporation gave an oil, which was treated with aqueous sodium hydroxide (2%; 5 ml). The mixture was warmed briefly to boiling and then set aside at room temperature for 15 min. After acidification with hydrochloric acid (conc.) to pH 2 and removal of most volatile material under reduced pressure, the semisolid residue was triturated with ether (1 × 25 ml). Evaporation left an oil (0·11 g) which was crystallised from heptane to give 2,5-dimethoxyphenylacetic acid, m.p. 121—123° (lit.,[7] 123°), $\nu_{max.}$ ($CHCl_3$) 3000vbr and 1720 cm^{-1}; δ [$(CD_3)_2CO$] 3·56 (2H, s), 3·72 (3H, s), 3·75 (3H, s), and 6·7—7·0 (3H, m).

Chromatographic fraction (i) was shown to be the lactone of 2-hydroxy-5-methoxyphenylacetic acid by comparison of its properties with those of synthesised material. Fraction (ii) consisted of the corresponding methyl ester (from the lactone during chromatography), identified by hydrolysis and comparison with 2-hydroxy-5-methoxyphenylacetic acid.

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