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SHORT COMMUNICATION

CYANOGENESIS IN MANIOC: LINAMARIN AND ISOLINAMARIN

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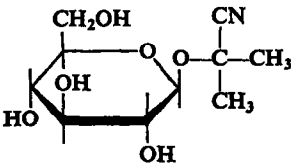
Abstract—The cyanogenetic glucoside linamarin was obtained in good yield from tubers of manioc (*Manihot esculenta* Crantz) by a chromatographic procedure. From the reaction of acetobromoglucose and acetone cyanohydrin both the α- and β-anomers of the glucoside of acetone cyanohydrin were obtained, and comparison of the properties of natural linamarin with those of the synthetic samples established the identity of the natural glucoside as the β-anomer. The spectroscopic properties of the α- and β-anomers are consistent with the configurational assignments.

INTRODUCTION

IN 1906, Dunstan, Henry and Auld[1] isolated a cyanogenetic glycoside from tubers of manioc (*Manihot esculenta Cranz*) and demonstrated that it was identical to linamarin, a cyanogenetic glycoside that had been isolated from flax seedlings (*Linum usitatissimum* L.) by Jorissen and Hairs.[2] Dunstan *et al.*, showed that linamarin was the glucoside of acetone cyanohydrin, and particularly because of its resistance to hydrolysis by such a β-glucosidase as emulsin, considered that it was an α-glucoside.[3] The latter conclusion was challenged,[4] however, and in 1919 Fischer and Anger[5] succeeded in synthesizing linamarin by a method that would be expected to afford a β-glucoside. The levorotatory properties of linamarin and of the intermediates in its synthesis supported the view that it was a β-glucoside.

Although the β-configuration for linamarin was thus strongly indicated, it was not rigorously established by the synthesis of both the α- and β-anomers. The weak hydrolytic activity of almond emulsin toward linamarin has been confirmed by various workers,[6] and because of this anomalous enzymatic behaviour, the question has remained of interest. Recently, Butler and co-workers[6] have presented some i.r. data as additional evidence for the β-configuration for linamarin. However, this spectral data, which will be discussed later, is



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[1] W. R. DUNSTAN, T. A. HENRY and S. J. M. AULD, *Proc. Roy. Soc.* **78**, 152 (1906).
[2] A. JORISSEN and E. HAIRS, *Bull. Acad. Roy. Sci. Belg.* **21**, 529 (1891).
[3] W. R. DUNSTAN, T. A. HENRY and S. J. M. AULD, *Proc. Roy. Soc.* **79**, 315 (1907).
[4] H. E. ARMSTRONG and E. HORTON, *Proc. Roy. Soc.* **82**, 349 (1910).
[5] E. FISCHER and G. ANGER, *Chem. Ber.* **52**, 854 (1919).
[6] G. W. BUTLER, R. W. BAILEY and L. D. KENNEDY, *Phytochem.* **4**, 369 (1965); and references cited therein.

not conclusive. In the present paper, the synthesis of the α-anomer of the glucoside of acetone cyanohydrin (isolinamarin) and its comparison with samples of both natural and synthetic linamarin are reported. In this way the β-configuration for the natural glucoside (structure I) is unequivocally demonstrated.

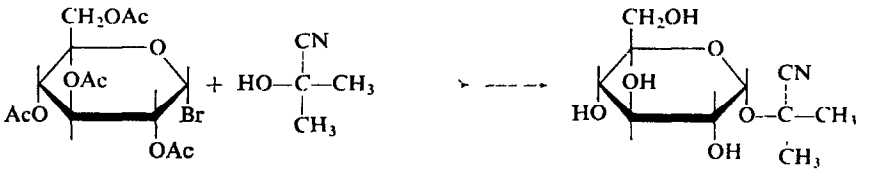
RESULTS AND DISCUSSION

Linamarin was isolated from the dried roots of "bitter" manioc by Dunstan *et al.*[1] after an attempt to obtain the glucoside from imported fresh roots was unsuccessful. Purification of the glucoside was achieved by successive precipitations from an alcoholic solution with ether, a process they recognized as "both tedious and wasteful". The difficulties generally encountered in the isolation of pure cyanogenetic glycosides have been pointed out by Dillemann,[7] who proposed that these difficulties account for the relatively small number of cyanogenetic species in which identity of the glycoside has been established. Thus Finnemore *et al.*[8] isolated only 1 per cent of the calculated quantity of lotaustralin, the glucoside of the cyanohydrin of methyl ethyl ketone, from dried white clover (*Trifolium repens* L.). An improved method for the isolation of dhurrin, the glucoside of *p*-hydroxy-L-mandelonitrile, that includes column chromatography on cellulose has recently been described.[9]

We have found that linamarin can be isolated expeditiously from tubers of manioc with a relatively low hydrogen cyanide content by column chromatography on silica gel, with chloroform–methanol as the eluting solvent. Column chromatography of the concentrate from an ethanolic extract, combined with treatment with activated carbon and crystallization from ethyl acetate, afforded a colorless crystalline sample of linamarin. The identity of this product to a sample synthesized from acetobromoglucose and ethyl α-hydroxyisobutyrate as starting materials by the method of Fischer and Anger[5] was demonstrated by mixed melting point, optical rotation, i.r. and NMR spectroscopy, and thin-layer chromatography.

A number of approaches to the synthesis of the α-glucoside of acetone cyanohydrin were tried and found to be unsatisfactory, apparently because of the difficulty in effecting the desired reactions with tertiary hydroxyl groups. Ultimately, the tetraacetate of the α-glucoside, together with a small quantity of the tetraacetate of linamarin, was obtained from the reaction of acetobromoglucose and acetone cyanohydrin without solvent in the presence of mercuric cyanide. Removal of the acetyl groups gave the α-glucoside of acetone cyanohydrin (II), isolinamarin. This anomer melted at 155–156° and had a specific rotation of +151°, as compared to a melting point of 143–144° and a rotation of −28·5° for the β-anomer, linamarin.

The NMR spectra of the α- and β-glucosides corroborate the assigned structures. The



II

[7] G. DILLEMANN, *Encyclopedia of Plant Physiology*, Vol. 8, p. 1056. Springer Verlag, Berlin (1958).
[8] H. FINNEMORE, J. M. COOPER and J. H. COBCROFT, *J. Soc. Chem. Ind.* **57**, 166 (1938).
[9] C.-H. MAO, J. P. BLOCHER, L. ANDERSON and D. C. SMITH, *Phytochem.* **4**, 297 (1965).

spectra of the natural and synthetic samples of linamarin contain a doublet at 5·15 τ that represents the anomeric proton, whereas it appears at 4·7 τ in the spectrum of isolinamarin. Such a location of this signal at lower field is characteristic of α-glucosides.[10] Further, the coupling constants for this doublet were found to be 7·2 c/s for linamarin and 3·2 c/s for isolinamarin, as would be anticipated for their respective formulations as β- and α-glucosides.[10] The NMR spectra of the tetraacetyl derivatives are also consistent with the assigned structures.

As mentioned previously, i.r. evidence for the β-configuration of linamarin has been reported by Butler *et al.*[6] These investigators advanced the absence of bands at 845 ± 9 cm^{-1} in the spectra of mixtures of linamarin and lotaustralin as evidence for the β-configuration of the glucosides, and they proposed the presence of a band in the 891 ± 7 cm^{-1} region as supporting, though inconclusive, evidence. In the 800–900 cm^{-1} region, the spectrum of pure linamarin shows a peak at 872 cm^{-1} and a weak band at 896 cm^{-1}. The spectrum of isolinamarin contains a peak at 888 cm^{-1} and rather weak, though definite, bands at 842 and 860 cm^{-1}. Thus the α-anomer is differentiated by the presence of absorption, though rather weak, in the 850 cm^{-1} region, but a band at 891 ± 7 cm^{-1} does not provide evidence for the β-configuration.

EXPERIMENTAL

Extraction of Manioc

Analysis of a 90-g sample of whole tubers of *Manihot esculenta*, harvested in Puerto Rico and transported to this Laboratory by air, by the method of Winkler[11] indicated a hydrogen cyanide content of 6·4 mg/100 g. Portions of 600 g of the whole tubers were cut into pieces and mixed for 2–3 min in a Waring Blendor with 1500 ml of 90% ethanol. After the resulting slurry had been heated to boiling for 10 min, it was filtered, and the filter cake was washed with two 100-ml portions of 90% ethanol. Concentration of the filtrate and washings in a rotary vacuum evaporator at 35–40°, followed by drying in a vacuum desiccator, afforded a viscous syrup. The 600-g portions yielded 20–22 g of concentrate.

Isolation of Linamarin [2-(β-D-*Glucopyranosyloxy*) *Isobutyronitrile*]

A 22·3-g portion of concentrate, obtained from 600 g of *Manihot*, was extracted with 170 ml of boiling methanol. The filtered solution was chromatographed on a column of 500 g of silica gel (Davison Co., Baltimore 3, Md., Grade 950, 60–200 mesh) with 5 : 1 chloroform–methanol as eluent. The fractions from the column were monitored for the presence of linamarin by thin-layer chromatography on plates of silica gel G (Stahl), developed with 5 : 1 chloroform–methanol, sprayed successively with a 2% solution of α-naphthol in ethanol and with conc. sulfuric acid, and heated. The fractions containing linamarin were combined in two portions. The first portion was concentrated in a rotary vacuum evaporator, and after the concentrate had been treated with 0·8 g of Darco G-60 in methanol, it was rechromatographed on a column of 60 g of silica gel, again with 5 : 1 chloroform–methanol as eluent. The fractions containing linamarin were concentrated, and the residue was crystallized from 9 ml of ethyl acetate. Linamarin separated as clumps of colorless crystals; 37·8 mg, m.p. 139–141°.

The second portion of pooled fractions from the 500-g column, which appeared to contain glucose as an impurity, was similarly concentrated, treated with Darco G-60, and

[10] R. U. Lemieux, *Chem. Can.* **16**, 14 (1964).
[11] W. O. Winkler, *J. Assoc. Offic. Agr. Chemists* **34**, 541 (1951).

rechromatographed on a column of silica gel (70 g). Crystallization from ethyl acetate gave 146 mg of colorless crystals, m.p. 141·5–142·5°. The total weight of linamarin obtained (183·8 mg) represents a 52 per cent yield of the calculated quantity in the 600 g of *Manihot*. Recrystallization from ethyl acetate afforded clusters of glistening crystals; m.p. 143–144°, $[\alpha]_D^{32} -28{\cdot}5°$ ($c = 3{\cdot}86$, water). A synthetic sample of linamarin gave m.p. 143–144° and $[\alpha]_D^{26} -27{\cdot}5°$. Specific rotations from −26 to −29° have been reported.[3,5,12]

Synthesis of Isolinamarin [2-(α-D-*Glucopyranosyloxy*) *Isobutyronitrile*]

A mixture of 5·12 g (0·0125 mole) of 2, 3, 4, 6-tetra-O-acetyl-α-D-glucopyranosyl bromide (acetobromoglucose) and 4·5 g (0·05 mole) of acetone cyanohydrin was stirred at 40–50°, and 3·12 g (0·0125 mole) of mercuric cyanide was added in small portions. Stirring was continued for 24 hr at 40–50° and then for 24 hr at room temperature. The mixture was poured into 100 ml of water, and after the precipitated oil had been separated, the aqueous solution was extracted with ether. The oil and ether extract were combined, dried over anhydrous sodium sulfate, and concentrated. The resulting yellow syrup (5·94 g) was chromatographed on a column of 300 g of Woelm silica gel. After a small quantity of dark material had been removed by elution with methylene chloride, elution with ethyl ether afforded a yellow oil that crystallized. Recrystallization from ether yielded 710 mg of 2-(2, 3, 4, 6-*tetra-O-acetyl-α-D-glucopyranosyloxy*) *isobutyronitrile* as colorless crystals, m.p. 108·5–109·5° $[\alpha]_D^{28} +24{\cdot}6°$ ($c = 0{\cdot}97$, acetone). (Found: C, 52·02; H, 6·19; N, 3·36%; mol. wt. by vapor pressure osmometer, 419. $C_{18}H_{25}O_{10}N$ required: C, 52·05; H, 6·07; N, 3·37%; mol. wt., 415.) For $[\alpha]_D^{14}$ of linamarin tetraacetate in acetone Fischer and Anger[5] report −10·66, −10·81°.

A second crystalline fraction was obtained by further elution of the silica gel column with ether. Recrystallization of this fraction from methanol yielded 35 mg of linamarin tetraacetate, m.p. 138–139°; lit.[5] m.p. 140–141°.

To a solution of 350 mg of isolinamarin tetraacetate in 4 ml of absolute methanol was added 3 mg of sodium methylate, and the solution was refluxed for 10 min. Removal of the solvent and crystallization of the concentrate from ethyl acetate–methanol afforded 109 mg of 2-(α-D-*glucopyranosyloxy*) *isobutyronitrile* as colorless crystals, m.p. 149·5–151°. Further crystallization from absolute ethanol–carbon tetrachloride gave an analytical sample, m.p. 155–156°, $[\alpha]_D^{25.5} +151°$ ($c = 3{\cdot}05$, water). (Found: C, 48·35; H, 7·04; N, 5·60. $C_{10}H_{17}O_6N$ required: C, 48·58; H, 6·93; N, 5·66%).

Spectra

The NMR spectra were recorded at 60 Mc on a Varian Model A-60 spectrometer. Deuterium oxide was used as a solvent for linamarin and isolinamarin, and carbon tetrachloride was used for the acetates. Infrared spectra were determined in potassium bromide pellets with a Beckman Model IR-12 spectrophotometer.

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[12] H. FINNEMORE, J. M. COOPER and M. B. STANLEY, *J. Soc. Chem. Ind.* **57**, 162 (1938).