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A NEW β-D-QUINOVOSIDE FROM COMMERCIAL *IPOMOEA PURGA*

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Key Word Index—*Ipomoea purga;* Convolvulaceae; Vera Cruz jalap; β-D-quinovoside of 11-hydroxytetradecanoic acid.

Abstract—Treatment of convolvulin (the ether insoluble portion of purified jalap resin) with sodium methoxide in methanol has yielded a new β-D-quinovoside. Periodate oxidation and methylation studies together with chemical and spectroscopic analyses have shown this fragment to consist of one molecule of D-quinovose glycosidically linked to a molecule of methyl 11-hydroxytetradecanoate.

INTRODUCTION

THE RESINOUS extracts obtained from certain plants of the Convolvulaceae have long been used for various medicinal purposes[1] but chiefly as powerful cathartic agents. Several reports have appeared in the literature concerning the chemical constituents of Jalap root (*Ipomoea purga* Vera Cruz jalap)[2–5] and related species.[6–14] The active constituents of the resins consist of complex glycolipids whose detailed structures are largely unknown. Votoček was the first to report the presence of D-quinovose in convolvulin (the ether-insoluble fraction of jalap resin). Stacey and Jones[5] have also isolated D-quinovose from jalap root. More recently, Shellard,[8] in his investigations of several species of the Convolvulaceae was unable to detect quinovose in convolvulin and has speculated that the material examined by Votecěk was not genuine convolvulin.

In this paper we report the isolation of D-quinovose present as the glycoside of 11-hydroxytetradecanoic acid. Legler[13] has also isolated a similar glycoside containing D-quinovose linked to 7-hydroxydecanoic acid from the leaves of *Ipomoea fistulosa.*

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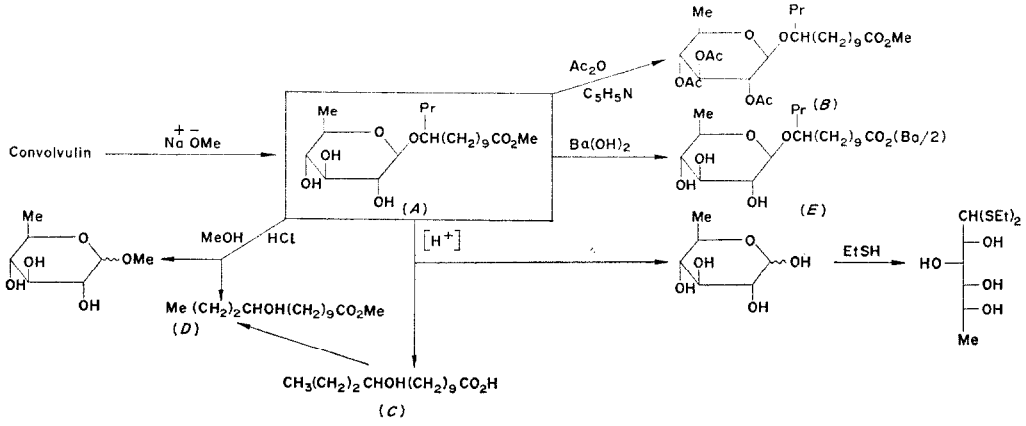
[1] SHELLARD, E. J. (1961) *Chem. Drug.* **176,** 219.
[2] HOEHNEL, W. (1896) *Arch. Pharmazie* **234,** 647, 670.
[3] VOTOCEK, E. (1910) *Ber. Deut. Chem. Ges* **43,** 476.
[4] POWER, F. B. and ROGERSON, H. (1910) *J. Am. Chem. Soc.* **32,** 80.
[5] STACEY, B. E. and JONES, J. K. N. unpublished results.
[6] POWER, F. B. and ROGERSON, H. (1908) *Am. J. Pharm.* **80,** 251.
[7] AUTERHOFF, H. and DEMLEITNER, H. (1955) *Arzneimittel-Forsch* **5,** 402.
[8] SHELLARD, E. J. (1961) *Planta Med.* **9,** 102, 141, 146.
[9] KHANNA, S. N. and GUPTA, P. C. (1967) *Phytochemistry* **6,** 735.
[10] SMITH, C. R. JR., NIECE, L. H., ZOBELL, H. F. and WOLFF, I. A. (1964) *Phytochemistry* **3,** 289.
[11] LEE, S. R., CHUNG, K. H. and KIM, H. S. (1968) *The Daehan Hwahak Hwoejee* **13,** 96.
[12] OKABE, H. and KAWASAKI, T. (1970) *Tetrahedron Letters* **36,** 3123.
[13] LEGLER, G. (1965) *Phytochemistry* **4,** 29.
[14] WAGNER, H. and KAZMAIER, P. (1971) *Tetrahedron Letters* **35,** 3233.

RESULTS AND DISCUSSION

Despite the extensive attention that convolvulin has received, its detailed structure remains unknown. The quinovoside described here was obtained by treating convolvulin with sodium methoxide, inferring that it is attached to the rest of the convolvulin molecule through an ester linkage. Furthermore, this ester link must involve the carboxyl group of the aglycone since methoxide treatment results in the isolation of the quinovoside in the form of its methyl ester. The quinovoside was also detected in salt form in the hydrolysate obtained on treating convolvulin with aqueous alkali. The overall identification scheme for the quinovoside is shown in Scheme 1.



SCHEME 1. IDENTIFICATION SCHEME FOR THE QUINOVOSIDE.

Continuous TLC on phosphate-impregnated Kieselgel[15] of the acid hydrolysate of the glycoside revealed quinovose to be the sole sugar component. The MW of the quinovoside (*A*) was determined in a number of ways: isopiestic, 360; MS, >330; saponification, 365; acetylation, 400; equivalent weight of the saponified quinovoside, 395.

These results indicated that only a single quinovose unit was present. Acetylation of the quinovoside gave a crystalline substance (*B*) whose IR spectrum showed moderate absorption at 11·2 μ indicating the existence of a β-glycosidic bond. The NMR spectrum also indicated a β-link since the C_5–Me resonance peak occurred at $\tau = 8{\cdot}80$ and $J = 6{\cdot}3$ Hz. The τ-values obtained by Sinclair and Sleeter[16] for methyl 2,3,4, tri-*O*-acetyl-6-deoxy-D-glucoside were 8·85 and 8·79 for the α- and β-anomers, respectively.

The aglycone and its methyl ester were identified by elemental analyses, comparison of their m.ps with literature values and from their MS. Thus, all of the expected ions[17] resulting from the usual cleavage points of hydroxy esters were readily detected in the MS of the aglycone ester (*D*). (Table 1).

Likewise the MS of the acetylated quinovoside (*B*) gave the characteristic fragmentation pattern identical to that reported[18] for peracetylated quinovose. Substance *B* consumed 4 equivalents of alkali when its acetyl content was determined by the method of Kunz.[19] This

[15] SINGH, S. and STACEY, B. E. (1972) *Analyst* **97**, 977.
[16] SINCLAIR, H. B. and SLEETER, R. T. (1970) *Tetrahedron Letters* **11**, 833.
[17] RYHAGE, R. and STENHAGEN, E. (1960) *Arkiv. Kemi.* **15**, 545.
[18] BIEMANN, K., DEJONGH, D. C. and SCHNOES, H. K. (1963) *J. Am. Chem. Soc.* **85**, 1763.
[19] WOLFROM, M. L. and THOMPSON, A. (1962) *Meth. Carbohyd. Chem.* **1**, 449.

result is consistent with the molecule having 3 acetyl groups and one other ester group. Further confirmation of the proposed structure (*A*) was provided by periodate oxidation and methylation. Periodate oxidation of (*A*) led to the consumption of 2·08 mol of periodate

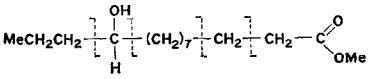
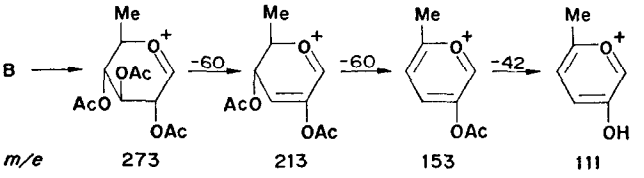


TABLE 1. MS DATA FOR THE METHYL ESTER OF AGLYCONE (SUBSTANCE *D*)

m/e	Relative abundance (%)	Ion	*m/e*	Relative abundance (%)	Ion
227	4·2	M-31	183	100	(215–32)
215	46	$CH(OH)(CH_2)_9CO_2Me$	87	100	$CH_2.CH_2.CO_2Me$
209	3·8	(227–18)	74	80	$MeCO_2Me$
186	85	$H.(CH_2)_9CO_2Me$	73	42	CH_2CO_2Me

and the release of 0·93 mol of formic acid corresponding to a 1,2,3-triol system. Methylation by the Purdie method[20] followed by methanolysis yielded methyl 2,3,4 tri-*O*-methyl-6-deoxy-D-glucoside as the only carbohydrate product.



EXPERIMENTAL

General. Solutions were concentrated below 40° under diminished pressure. M.ps were determined on a Kofler block and are uncorrected. NMR spectra were obtained in CCl_4 and D_2O. MW determinations were carried out using a Perkin-Elmer 115 Molecular Weight Apparatus in C_6H_6 or EtOH–H_2O (4:1).

Chromatography. For purification purposes dry column chromatography using Kieselgel 7734 (Merck) was employed with the following eluants: (I) $CHCl_3$–MeOH (4:1); (IIa) C_6H_6–*iso*-PrOH (5:1); (IIb) C_6H_6–*iso*-PrOH (10:1); (IIIa) EtOAc–EtOH–H_2O (20:8:7); (IIIb) EtOAc–EtOH–H_2O (10:3:2); (IV) C_6H_6–Et_2O–HCO_2H (30:15:1). For monitoring and identification purposes TLC was performed using Kieselgel 7739 (Merck) and the above eluants except for the identification of quinovose when Kieselgel 7736 buffered with Na_2HPO_4 was employed with Me_2CO–MeOH–$CHCl_3$–H_2O (10:4:2:1) as eluant.[15]

Spray reagents. (1) 5–10% H_2SO_4 in EtOH. The sprayed plates were heated to produce charred spots. (2) 0·5% $KMnO_4$ in 1 M NaOH. (3) 3% w/v *p*-Anisidine hydrochloride in BuOH–EtOH–H_2O (5:1:1). The sprayed paper or plate was heated at 110° for 15 min.

Materials. Dried jalap root was supplied by Berk Ltd., London, and its authenticity was confirmed by Dr. W. E. Court (University of Bradford).

Isolation of convolvulin. The dried root was crushed and ground to a coarse powder and extracted with boiling EtOH for 6 hr. Most of the solvent was removed and the soft resin was poured into a large vol. H_2O. The precipitated resin was redissolved in EtOH, decolourized with charcoal, and after removal of the solvent the residue was repeatedly extracted with dry Et_2O. The Et_2O-insoluble resin was further purified by column chromatography by eluting with solvent (I). Removal of solvent from the appropriate fractions of the eluate and vacuum drying at 40° gave an almost white, amorphous, hygroscopic powder m.p. ≃ 163° and showing a single spot (R_f 0·4) when examined by TLC using solvent (IIIa). Elemental analyses (C, 52·7%; H, 7·93%) gave the empirical formula $C_9H_{16}O_5$.

[20] HIRST, E. L. and PERCIVAL, E. (1965) *Meth. Carbohyd. Chem.* **5**, 294.

Isolation of the quinovoside (A). 10 g batches of purified convolvulin in MeOH (100 ml) were treated with Na (0·2 g) in MeOH (50 ml) and stood overnight at room temp. This mixture gave 4 main spots (R_fs 0·15, 0·27, 0·45, 0·75) by TLC using solvent (IIIa). Concentration of the mixture to a thick syrup followed by shaking with dry Et_2O (200 ml) yielded a large quantity of white. ppt. Filtration and TLC examination (solvent IIIa) of the ppt and of the ethereal solution showed the latter to consist almost exclusively of the fastest component (R_f 0·75) whilst the ppt contained the 3 slower moving components. The ethereal solution was concentrated and purified by column chromatography using solvent IIa to give a colourless, low m.p. (below 30°) solid (0·8 g). Several attempts were made to recrystallize this material but with only partial success. Crystallization did occur below 15° but due to the low m.p. and its affinity for H_2O the quinovoside tended to soften at room temp. This product was quite pure and gave negative results for acidity and with Fehling's and Tollen's reagents but gave a positive hydroxamic acid test.

Acetylation. The quinovoside (260 mg) was acetylated with excess Ac_2O (5 ml) in dry pyridine (10 ml) overnight and the product (190 mg) isolated by pouring the mixture into ice-water. Recrystallization (×5) from cyclohexane gave needles, m.p. 75–76°. Quantitative acetylation was carried out by the method of Fritz and Shenck.[21] Hydroxyl content (Calc. for $C_{21}H_{37}O_4(OH)_3$: 12·2%, Found: 12·1%).

Saponification. 76·5 mg of *A* consumed 0·21 mmol of alkali on refluxing with standard 0·1 M NaOH in 90% EtOH; that is, the equivalent weight of the quinovoside was 365.

Preparation of the barium salt. The quinovoside (0·7 g) in MeOH (20 ml) was treated with saturated $Ba(OH)_2$ (8 ml) at room temp. After 48 hr TLC showed that the starting material had been completely hydrolysed. The solvent was removed and the residue treated with MeOH (50 ml) and a small amount of insoluble residue was removed. Excess CO_2 was bubbled in and a further ppt was filtered off. Evaporation of the filtrate gave a solid (0·65 g) which was free from the starting material and showed only one component by TLC (solvent IIIb). 41·8 mg of this product gave 10·9 mg of $BaSO_4$ on heating with H_2SO_4, inferring a value of 380 for the equivalent weight of the saponified acid or a value of 394 for the quinovoside (*A*).

Acid hydrolysis. (*A*) (0·8 g) was refluxed with 30 ml of Kiliani solution [*c* HCl (10 ml) + HOAc (35 ml) + H_2O (55 ml)] for 4 hr and the hydrolysate extracted with Et_2O (3 × 30 ml). The aq. layers was reserved for the examination of the carbohydrate components. The etheral extracts were shaken with satd. $NaHCO_3$ the aq. layer was acidified with dil. HCl and the aglycone re-extracted into Et_2O.

Examination of the aglycone. The above ethereal solution containing the aglycone was evaporated to dryness and purified by column chromatography using solvent (IV) to give a solid (280 mg), m.p. 49° (lit.[13] 49–50° for 11-hydroxytetradecanoic acid) (*Anal.* Calc. for $C_{14}H_{28}O_3$: C, 68·9%; H, 11·5%. Found: C, 69·0%; H, 11·5%). The IR spectrum showed strong absorption at 3·0 μ (OH) and 5·90 μ (C=O).

Preparation of the methyl ester (D). The aglycone (200 mg) was refluxed with 10 ml of BF_3–MeOH reagent (about 14%) for 1 hr and the cooled mixture was extracted with pentane (3 × 20 ml). The combined pentane extracts were successively washed with aq. alkali and H_2O before the removal of solvent and recrystallization to give the methyl ester (150 mg) m.p. 29° (lit.[13] 30–31°) (*Anal.* Calc. for $C_{15}H_{30}O_3$: C, 69·8%; H, 11·6%. Found: C, 69·7%; H, 11·5%).

Examination of the carbohydrate component. The aq. layer containing the sugar components obtained on acid hydrolysis was evaporated to dryness with several portions of absolute EtOH. The residue was examined by continuous TLC[15] and by PC (borate buffer)[22] and found to consist of pure quinovose. Its NMR spectrum in deuterium oxide was identical to the spectrum obtained from authentic D-quinovose.

Preparation of the diethyl dithioacetal derivative. The above sugar (50 mg) was dissolved in HCl (0·25 ml) at 0°, mixed with ethanethiol (0·2 ml) and stirred for 15 min at 0°. The mixture was extracted with $CHCl_3$ (4 × 5 ml) and the combined $CHCl_3$ extracts, after washing with H_2O (2 ml) were dried. Concentration and subsequent recrystallization of the solid from hexane–$CHCl_3$ (3:1) yielded pure D-quinovose diethyl dithioacetal (20 mg), m.p. 97–98° (lit.[23] 97–98°), m.m.p. 97°; $[\alpha]_D^{20}$ −50° (*c* 1·00, H_2O); lit.[23] +47·1° (*c* 1·0, H_2O) for the L-quinovose derivative.

Methanolysis of (A). *A* (0·5 g) was dissolved in dry MeOH saturated with HCl gas (10 ml) and kept at room temp. overnight. Evaporation followed by extraction with Et_2O and column chromatography (solvent IV) gave a crystalline sample (270 mg) which was identical in all respects to substance (*D*) synthesized above. The Et_2O-insoluble residue (140 mg) was purified by column chromatography using solvent (IIIb) to give a solid (90 mg). This was identified as methyl quinovoside by TLC and by acid hydrolysis when quinovose was the only monosaccharide produced.

Periodate oxidation. (*A*) (280 mg) contained in a 100 ml flask was dissolved in *t*-BuOH-H_2O (1:1) (70 ml) and $NaIO_4$ (0·1 M, 25 ml) added and made up to vol. with the above solvent. The flask was kept below 5° and aliquots (5 ml), withdrawn at intervals, were mixed with a solution (40 ml) containing KI (0·4 g) and N H_2SO_4 (1·5 ml). The liberated I_2 was immediately titrated with 0·1 M $Na_2S_2O_3$. A concurrent blank determination was carried out. For the estimation of HCO_2H produced aliquots (5 ml) of the reaction mixture

[21] FRITZ, J. S. and SCHENK, G. H. (1959) *Anal. Chem.* **31**, 1808.
[22] KRAUSS, M. T., JAGER, H., SCHINDLER, O. and REICHSTEIN, T. (1960) *J. Chromatog.* **3**, 63.
[23] ZISSIS, E., RICHTMEYER, N. K. and HUDSON, C. S. (1951) *J. Am. Chem. Soc.* **73**, 4714.

were treated with acid-free ethanediol (1 ml) and, after 20 min, titrated with 0·02 M NaOH. Each mole of the quinovoside consumed 2·08 mol periodate and simultaneously released 0·93 mol HCO_2H

Methylation. (*A*) (0·3 g) was 2× methylated by the modified Purdie method[20] in $HCONMe_2$. Following cyanide extraction, the product was methanolysed at room temp. overnight. During evaporation under diminished pressure a crystalline substance, m.p. 59°, sublimed close to the neck of the flask and was identified as methyl 2,3,4-tri-*O*-methyl-6-deoxy-D-glucoside from its chromatographic mobility and analysis (*Anal.* Calc. for $C_{10}H_{20}O_5$: C, 54·6%; H, 9·1%. Found: C, 54·4%; H, 9·0%).

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