Phytochemistry, 1964, Vol. 3, pp. 289 to 299. Pergamon Press Ltd. Printed in England

GLYCOSIDIC CONSTITUENTS OF *IPOMOEA PARASITICA* SEED

C. R. Smith, Jr., L. H. Niece,* H. F. Zobel and I. A. Wolff
Northern Regional Research Laboratory,† Peoria, Illinois

(*Received* 29 *August* 1963)

Abstract—The seeds of *Ipomoea parasitica* (HBK) Don. contain unique members of a class of glycolipids found in the plant family Convolvulaceae. They were shown to consist of acylated glycosides of (+)-11-hydroxyhexadecanoic acid. The acyl groups were removed by alkaline hydrolysis and obtained as α-methylbutyric acid. Three sugars were liberated by acid hydrolysis: D-fucose, 6-deoxy-D-glucose (D-quinovose), and another tentatively identified as a 6-deoxygulose. These glycosides differ from others found in the Convolvulaceae in yielding this tentatively identified sugar, and also in containing neither D-glucose nor L-rhamnose. It was shown for the first time that different sugars can be released quite selectively from a convolvulaceous glycoside by graded acid hydrolysis. On the basis of these hydrolyses, a tentative structure for the glycosidic units is proposed.

A DISTINCTIVE group of complex glycosides is widely distributed among members of the plant family Convolvulaceae.[1,2] These glycosides may occur in different parts of the convolvulaceous plants—in tubers, stems or seeds. They vary considerably in the components they yield on complete hydrolysis, but they have definite unifying features. On alkaline hydrolysis, they yield short-chain aliphatic acids plus glycosidic acids. When subjected to acid hydrolysis, these glycosidic acids in turn yield hydroxylated fatty acids as aglycons in addition to aldohexose sugars.[3,4] The sugars most often obtained on hydrolysis of the convolvulaceous glycosides have been D-glucose, L-rhamnose, and D-fucose (rhodeose).[3,4] 6-Deoxy-D-glucose (isorhodeose; D-quinovose) also has been reported at least once.[5]

The aglycons most frequently obtained from convolvulaceous glycosidic acids have been (+)-11-hydroxyhexadecanoic acid (jalapinolic acid)[6] and convolvulinolic acid—originally described as a C_{15}-compound,[7] but later proved to be 11-hydroxytetradecanoic acid.[8] Dihydroxy derivatives of tetradecanoic,[9] hexadecanoic,[10] and octadecanoic[11] acids, referred to in some cases by trivial names, also have been obtained from these substances as aglycons.

Convolvulaceous glycosides occur as mixtures of components that can be separated by means of selective solvent extractions.[3,4] Relatively little has been established concerning

---

* Present address, Department of Chemistry, University of Missouri, Columbia, Missouri.

† A laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

[1] A. Tschirch and E. Stock, *Die Harze*, Vol. II, part 2, p. 1618, Gebrüder Bornträger, Berlin (1936).
[2] E. J. Shellard, *Chemist and Druggist* **176**, 219 (1961).
[3] H. Auterhoff and H. Demleitner, *Arzneimittel-Forsch.* **5**, 402 (1955), and references cited therein.
[4] E. J. Shellard, *Planta Med.* **9**, 102, 141, 146 (1961), and references cited therein.
[5] E. Votoček, *Ber. Deut. Chem. Ges.* **43**, 476 (1910).
[6] L. A. Davies and R. Adams, *J. Amer. Chem. Soc.* **50**, 1749 (1928).
[7] Y. Asahina and M. Akasu, *J. Pharm. Soc. Japan*, No. **523**, 779 (1925); *C.A.* **20**, 365 (1926).
[8] T. Kawasaki, *J. Pharm. Soc. Japan* **70**, 485 (1950).
[9] Y. Asahina and S. Nakanishi, *J. Pharm. Soc. Japan* No. **520**, 515 (1925); *C.A.* **19**, 3479 (1925).
[10] E. Votoček and V. Prelog, *Coll. Czech. Chem. Comm.* **1**, 55 (1929).
[11] A. L. Misra and J. D. Tewari, *J. Indian Chem. Soc.* **30**, 391 (1953), and references therein.

their finer points of structure. Bauer and Junge[12] obtained some evidence as to the sequence of sugar units in scammonic acid, a product of the alkaline hydrolysis of scammony resin. Using a crude emulsin preparation, they partially degraded this tetrasaccharide and surmised the sequence:

D-glucose → D-glucose → L-rhamnose → D-fucose → 11-hydroxyhexadecanoic acid

Jaretzky and Risse[13] later tried to repeat this work, with negative results; their enzyme preparation was more highly purified and, therefore, probably more selective than the one used by Bauer and Junge. The carboxyl groups of the aglycon acids are evidently esterified in some way.[3,14] On the basis of the colloidal properties of jalap resin, Mannich and Schumann[14] postulated a polymeric structure in which the carboxyl of one glycosidic unit was esterified with a hydroxyl on a sugar of a similar unit, thus forming a polyester-type chain. In contrast, Misra and Tewari stated that the glucosidic acid constituent of *Ipomoea muricata* seed was present as an ethyl ester.[11] The experimental basis for this observation was not made clear.

Evidently it has not been recognized that certain glycolipid bacterial metabolites that have been discovered comparatively recently are strikingly similar to the glycosides of the Convolvulaceae in their general makeup.[15,16] A rhamnolipid produced by *Pseudomonas aeruginosa* also bears some resemblance to the glycolipids of the Convolvulaceae.[17]

The present paper is concerned with the characterization of the previously uninvestigated glycosides of *Ipomoea parasitica* seed.

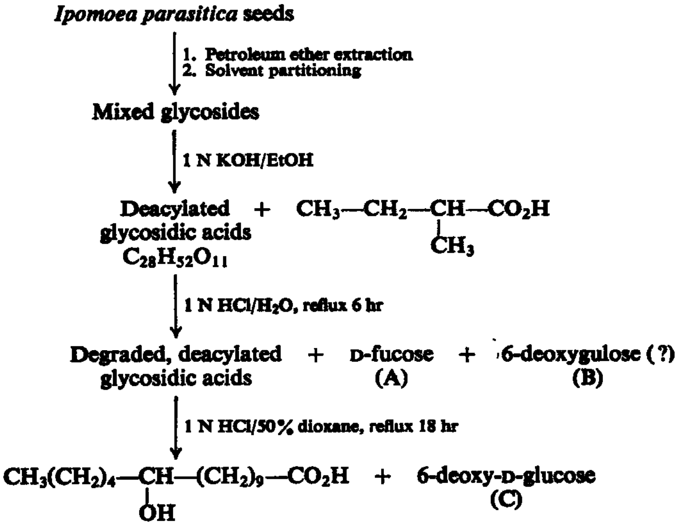
CHARACTERIZATION OF *IPOMOEA PARASITICA* SEED GLYCOSIDES

The glycolipid constituents obtained in the petroleum ether extract of *Ipomoea parasitica* seed were readily separated from the accompanying triglyceride oil by partitioning the mixture between hexane and a polar solvent, e.g. 90% ethanol. The polar phase yielded a resinous material whose i.r. spectrum showed very strong hydrogen-bonded hydroxyl absorption (2·9 μ), moderate ester or other carbonyl (5·78 μ), and a very strong peak (9·35 μ) suggesting ether linkages.[18] Thin-layer chromatography suggested that the material was a mixture of at least four components. These were only partially resolved by a 30-stage countercurrent distribution. Comparison of the i.r. spectra of selected fractions from this distribution indicated that these components differed in their relative content of ester and free hydroxyl groups.

Hydrolysis of the unfractionated glycoside mixture with ethanolic potassium hydroxide yielded a glycosidic acid (or acids) as the main product. A steam-volatile acid fraction was also obtained, which was shown by gas–liquid chromatography to consist mainly of a branched C_5-acid. This was identified as D- or L-α-methylbutyric acid by application of the permanganate degradation technique suggested by Murray.[19] The formation of other low-molecular weight products (e.g. water-soluble alcohols) in the alkaline hydrolysis was not excluded.

[12] K. H. BAUER and R. JUNGE, *Arch. Pharm.* **272**, 841 (1934).
[13] R. JARETZKY and E. RISSE, *Arch. Pharm.* **278**, 379 (1940).
[14] C. MANNICH and P. SCHUMANN, *Arch. Pharm.* **276**, 211 (1938).
[15] R. U. LEMIEUX, *Canad. J. Chem.* **29**, 415 (1951).
[16] P. A. J. GORIN, J. F. T. SPENCER and A. P. TULLOCH, *Canad. J. Chem.* **39**, 846 (1961).
[17] W. R. JARVIS and J. R. JOHNSON, *J. Amer. Chem. Soc.* **71**, 4124 (1949).
[18] L. J. BELLAMY, *The Infra-red Spectra of Complex Molecules*, p. 115, John Wiley, New York (1958).
[19] K. E. MURRAY, *Australian J. Chem.* **12**, 657 (1959).

Elementary analyses of the deacylated glycoside fraction indicated the empirical composition $C_{28}H_{52}O_{11}$. Approximately two moles of periodate were consumed per equivalent of glycoside, calculated on the basis of this formula—one rapidly, the second much more slowly. The neutral equivalent did not indicate that this acidic glycoside contained more than one such unit. Acid hydrolysis ultimately split this glycosidic acid fraction into a hydroxy acid and three sugars. However, a good deal of difference was found among the three glycosidically bound sugars in their susceptibility to acid-catalyzed hydrolysis. One sugar fraction was obtained free by treating the deacylated glycoside, for example, with 1 N aqueous hydrochloric acid at reflux for 6 hr; it was accompanied by a chloroform-soluble product found to be partially degraded glycoside. Practically no sugar-free aglycon was obtained under these conditions. This acid-labile sugar fraction was liberated under even milder conditions, e.g. by allowing the deacylated glycoside to stand in *ca.* 1 N hydrochloric acid at room temperature for 2 hr. In contrast, rather different and fairly rigorous conditions were required to complete the hydrolysis of the partially degraded glycoside—refluxing with 1 N methanolic hydrochloric acid or with 1 N hydrochloric acid in 50% aqueous dioxane for 18 hr.



The sugar fraction liberated under mild conditions was shown to be a mixture of approximately equal amounts of two sugars (A and B) by paper chromatography with the solvent system ethyl acetate–pyridine–water (120:50:40).[20] Chromatography of the more difficultly hydrolysable sugar fraction gave a single spot (Sugar C) having an R_f almost identical with that of B (the faster moving of the acid-labile sugars), and also with the R_f of L-rhamnose. The observed R_f values as well as the Dische–Shettles[21] and vanillin–perchloric acid[22] color reactions indicated that all the sugars were 6-deoxyaldohexoses. Further paper chromatographic studies, in which solvent systems V and VII of Krauss *et al.*[23] were used, suggested that Sugars B and C were different from L-rhamnose; preparation of their diethyl dithioacetals proved this, and also showed that they differed from each other. Sugars A and B were found

---

[20] I. Smith, *Chromatographic Techniques*, pp. 166, 169, Interscience Publishers, New York (1958).
[21] Z. Dische and J. B. Shettles, *J. Biol. Chem.* 175, 595 (1948).
[22] A. P. MacLennan, H. M. Randall and D. W. Smith, *Anal. Chem.* 31, 2020 (1959).
[23] M. T. Krauss, H. Jäger, O. Schindler and T. Reichstein, *J. Chromatog.* 3, 63 (1960).

only in the acid-labile sugar fraction, and Sugar C was obtained *only* from the acid-resistant core of the glycoside.

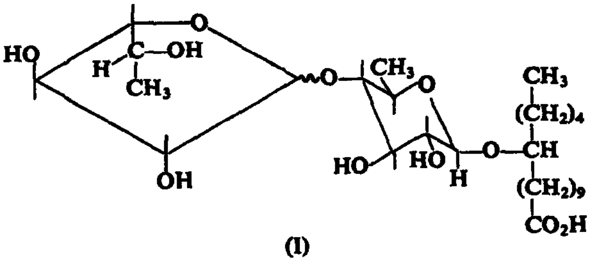
Sugar A was shown to be D-fucose by comparison of the melting point and X-ray diffraction pattern of its diethyl dithioacetal with those of the corresponding derivative of its optical antipode, L-fucose. Sugar C was similarly characterized as 6-deoxy-D-glucose (D-quinovose) by means of its diethyl dithioacetal. Sugar B yielded a diethyl dithioacetal whose melting point (147–147·5°) differed from any recorded for a 6-deoxyhexose to our knowledge. The R_f values observed in paper chromatography pointed to one of the enantiomers of 6-deoxyallose or 6-deoxygulose as the most likely possibility for Sugar B. 6-Deoxy-D- and L-allose were largely eliminated as possibilities by preparation of the diethyl dithioacetal of the D-isomer, m.p. 99–100°. The diethyl dithioacetal having m.p. 99–100° and the one having m.p. 147–147·5° could not be interconverted by cross-seeding experiments, and so are not likely to be polymorphic forms of the same compound. Sugar B is tentatively considered to be either the D- or L-isomer of 6-deoxygulose. Unfortunately a sample of the diethyl dithioacetals of neither was available for comparison.

The aglycon was obtained either as a crystalline methyl ester or free acid, depending on the method of hydrolysis of the degraded glycoside. Infrared spectra and gas chromatographic analyses indicated that the aglycon was mostly a C_{16}-monohydroxy acid. Oxidative cleavage of the methyl ester with chromium trioxide–acetic acid yielded pentanoic and hexanoic acids, together with half esters of undecanedioic and dodecanedioic acids. These fragments located the hydroxyl at C_{11} of a normal C_{16} carbon chain. The melting points of the free acid and its methyl ester, together with the optical rotation of the ester, indicated that the aglycon is (+)-11-hydroxyhexadecanoic acid and very probably is identical with the jalapinolic acid characterized by Davies and Adams.[6]

DISCUSSION

The ultimate hydrolysis products of *Ipomoea parasitica* seed glycosides can be related to one another within the frame of reference of findings on related glycosides. It seems reasonable to assume that the α-methylbutyric acid is present as acyl groups on sugar hydroxyls. The i.r. spectra of the various glycoside fractions and degradation products support this assumption, but other possibilities are not ruled out by our results. The low acid value of the seed extract indicates most of the carboxyl groups of the aglycon must be esterified in some manner. Apparently they are esterified with hydroxyls of sugar units, as in convolvulinic acid according to the findings of Mannich and Schumann.[14] The elementary analyses support the conclusion that the deacylated glycosidic fraction is a mixture of disaccharides of hydroxyhexadecanoic acid having the empirical formula $C_{28}H_{52}O_{11}$. Other glycoside species (e.g. mono- and trisaccharides) could possibly be present in combinations such as to give this empirical composition fortuitously. Formula I accommodates the other experimental observations on the deacylated glycosides—not uniquely, but as a likely possibility for one species present. D-Fucose and Sugar B are combined in the glycosides exclusively by very acid-labile linkages and therefore must be end units in the disaccharides. The end unit is depicted as D-fucose in I, but an analogous structure having a 6-deoxygulose as the terminal sugar would also be appropriate. Since 6-deoxy-D-glucose is obtained only from the more acid-resistant portion of the molecule, it must be joined directly to the hydroxyhexadecanoate moiety. Apparently this highly selective release of the sugars in convolvulaceous glycosides by graded acid hydrolysis has not been observed previously.

In view of the greater stability of glucopyranosides compared to glucofuranosides,[24, 25] the 6-deoxy-D-glucose unit must be in the pyranose form. The maximum at 11·23 μ in the i.r. spectrum of the degraded glycoside indicates an axial hydrogen at C–1 (β-D-glucosidic linkage).[26] In contrast to the large differences in hydrolysis rate between pyranosides and furanosides, the difference in hydrolysis rate between anomeric glycosides with the same ring form is small.[25, 27, 28] The very bulky secondary alkyl substituent in the glycoside from *Ipomoea* probably causes steric hindrance to the protonation stage involved in the hydrolysis. As depicted in I, the glucopyranoside ring has the preferred chair conformation (*C1* in Reeves' notation[29]) having all substituents equatorially oriented.



(I)

The ease of hydrolysis of the terminal sugars suggests that they are in the furanoside form.[25, 30] The observed consumption of two moles of periodate would permit them to be joined to the glucopyranoside ring by the common 1→4 linkage, as in I, or by a 1→2 linkage, rarely encountered in nature. This linkage might then be either α or β. It has been shown that furanosides having a *cis* relationship of substituents at C–1 and C–2 are more easily hydrolyzed under acidic conditions than those having these substituents *trans*.[31] The differences are relatively small, however, and afford no basis for a choice between an α or β furanoside linkage in Formula I. There is also the possibility of a 1→3 linkage between the two sugar units; this would require a *pyranoside* ring for the terminal sugar to accommodate two moles uptake of periodate.

The glycosides of *Ipomoea parasitica* resemble a number of other such constituents of convolvulaceous plants that have been studied. They are unique, however, in the nature of their sugar constituents in containing neither D-glucose nor L-rhamnose. They would represent the first example of glycosides of this group that contain a 6-deoxygulose. 6-Deoxy-D-gulose has been found in nature only as a constituent of a few cardiac glycosides;[32–34] the L-isomer has not been encountered.

---

[24] W. N. Haworth and E. L. Hirst, *J. Chem. Soc.* 2615 (1930).

[25] F. Shafizadeh in *Advances in Carbohydrate Chemistry* (Edited by M. L. Wolfrom), Vol. 13, p. 9, Academic Press, New York (1958).

[26] S. A. Barker, E. J. Bourne, M. Stacey and D. H. Whiffen, *J. Chem. Soc.* 171 (1954).

[27] R. Bentley, *Nature, Lond.* **176**, 870 (1950).

[28] J. T. Edward, *Chem. and Ind.* 1102 (1955).

[29] R. L. Reeves, *J. Am. Chem. Soc.* **72**, 1949 (1950).

[30] H. C. Brown, J. H. Brewster and H. Shechter, *J. Amer. Chem. Soc.* **76**, 467 (1954).

[31] I. Augestad and E. Berner, *Acta Chem. Scand.* **10**, 911 (1956).

[32] K. Doebel, E. Schlittler and T. Reichstein, *Helv. Chim. Acta* **31**, 688 (1948).

[33] W. Nagata, Ch. Tamm and T. Reichstein in *Festschr. Arthur Stoll*, p. 715, Birkhäuser AG, Basel (1957).

[34] R. Rees, C. R. Gavilanes, W. Meier, A. Fürst, K. Meyer and T. Reichstein, *Helv. Chim. Acta* **44**, 1607 (1961).

EXPERIMENTAL

Melting points were determined with a Fisher–Johns block[35] and are uncorrected. Infrared spectra were determined with an Infracord Model 137 spectrophotometer. Gas chromatographic analyses were carried out with a Burrell Kroma-tog K–5 instrument as described by Miwa and co-workers,[36] except that free acids were analyzed in some cases instead of methyl esters.

Isolation of Glycosides

Coarsely-ground seeds (81·2 g) of *Ipomoea parasitica* (HBK) Don. were extracted overnight in a Soxhlet apparatus with 30–60° petroleum ether. The solvent was removed *in vacuo*, leaving 13·8 g of light yellow extract having a gelatinous consistency; free acid content (calculated as C_{18} acid), 2·3%. A 9·30-g portion of this extract was partitioned between petroleum ether and 90% ethanol in a separatory funnel. The combined petroleum ether layers were dried over sodium sulfate and evaporated; 6·05 g of fluid oily material thus obtained had an i.r. spectrum typical of ordinary unsaturated glycerides. The combined alcoholic layers, upon evaporation, yielded 3·50 g of resinous glycosidic material whose i.r. spectrum (in 1% chloroform solution) included maxima at 2·90 μ (very strong, broad); 5·78 μ (strong) and 9·35 μ (very strong, broad—ether linkage[18]). Thin-layer chromatographic analyses of the glycosidic material were carried out with plates prepared as described by Stahl,[37] using the solvent system chloroform–methanol (9:1)[38] and 50% sulfuric acid spray.[39] The presence of three or four components was indicated.

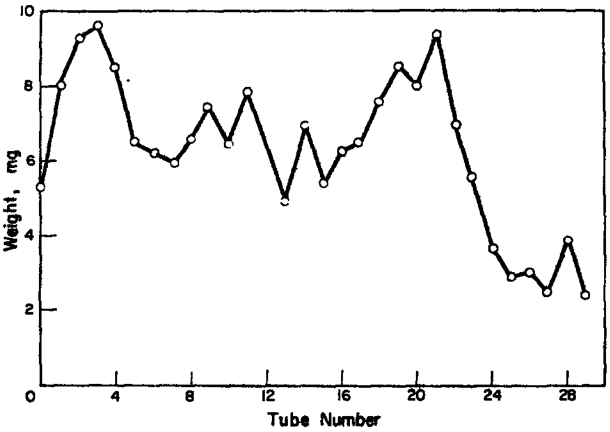


FIG. 1. COUNTERCURRENT DISTRIBUTION OF *Ipomoea parasitica* GLYCOSIDES.

Countercurrent Distribution of Glycosides

A 0·19-g portion of the glycosidic material was subjected to a 30-tube countercurrent distribution in the solvent system 95% ethanol–water–hexane–chloroform (7:7:12:2). The

---

[35] The mention of trade names or products does not constitute endorsement by the Department of Agriculture over those not named.

[36] T. K. MIWA, K. L. MIKOLAJCZAK, F. R. EARLE and I. A. WOLFF, *Anal. Chem.* 32, 1739 (1960).

[37] E. STAHL, G. SCHRÖTER, G. KRAFT, and R. RENZ, *Pharmazie* 11, 633 (1956).

[38] H. WAGNER, *Fette Seifen Anstrichmittel* 62, 1115 (1961).

[39] L. J. MORRIS, R. T. HOLMAN and K. FONTELL, *J. Lipid Research* 2, 68 (1961).

material was widely distributed through the tubes, as indicated in Fig. 1. Thin-layer chromatographic analyses of material from selected tubes indicated only partial separation of at least 4 components with chromatographic mobilities corresponding to their countercurrent mobilities—having R_f values of 0·25, 0·63, 0·73 and 0·83 in chloroform–methanol (9 : 1). The i.r. spectra of materials from tubes 3 and 20 resembled that of the starting mixture but differed considerably in the relative intensities of OH and C=O peaks (tube 3 had more OH, tube 20 more carbonyl).

Isolation of Deacylated Glycoside

Petroleum ether extract of *Ipomoea parasitica* seed (33·4 g) was refluxed under nitrogen 1 hr with 600 ml of 1 N ethanolic potassium hydroxide. The cooled mixture was diluted with 1200 ml of water and then extracted with (5 × 200 ml) portions of ethyl ether. The combined ether extracts were, in turn, washed with water; the aqueous washings and the alkaline liquor were combined. The ether layer was dried with sodium sulfate and evaporated, and 1·02 g of unsaponifiables was obtained. The alkaline liquor was acidified with hydrochloric acid, saturated with sodium chloride, and immediately extracted with ethyl ether. The ether extracts, after being dried with sodium sulfate and evaporated, yielded 31·7 g of acidic material having a sharp odor. A 1·02-g portion of these acids was partitioned between acetonitrile and hexane in a separatory funnel. The acetonitrile layer was re-extracted with fresh portions of hexane. Combined hexane layers yielded upon evaporation 0·67 g of free acids. The acetonitrile layer yielded 0·25 g of *deacylated glycoside*; this gave negative tests (sodium fusion) for nitrogen, sulfur, phosphorus, and halogens.[40] Determinations of neutral equivalent gave rather erratic results and suitable conditions for obtaining good replicability were not established. There were strong i.r. maxima at 2·90 μ, 5·82 μ and 9·35 μ; there was no selective absorption in the u.v. region. (Found: C, 59·7; H, 9·3; neutral equivalent, 612. $C_{28}H_{52}O_{11}$ requires: C, 59·6; H, 9·3; neutral equivalent, 567.)

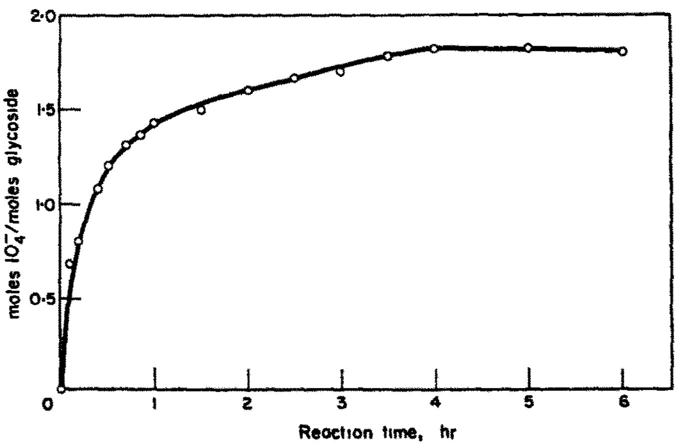


FIG. 2. COURSE OF THE REACTION OF PERIODIC ACID WITH DEACYLATED GLYCOSIDE FROM *Ipomoea parasitica*.

Quantitative periodate oxidation of the deacylated glycoside was carried out by the

---

[40] R. L. SHRINER, R. C. FUSON and D. Y. CURTIN, *Systematic Identification of Organic Compounds*, 4th Edition, p. 57, John Wiley, New York (1956).

procedure of Jackson and Hudson[41] somewhat modified. An 0·915-g portion of deacylated glycoside was dissolved in 155 ml of 50% *t*-butanol; 40 ml of 0·1 M periodic acid was added. A blank solution was prepared simultaneously. 5-ml aliquots of each were withdrawn at various intervals of time, mixed with 12 ml of 0·0152 M arsenious acid, 4 ml of saturated sodium bicarbonate, and 1 ml of 20% potassium iodide solution in saturated bicarbonate. The aliquots were then cooled over ice and titrated with 0·0151 M iodine to the starch end-point. Approximately 1·8 moles of periodate per mole of substrate was consumed. Results are summarized in Fig. 2.

Alkaline Hydrolysis of Glycosides and Isolation of Hydrolysis Products

A 3·3-g portion of the mixture of glycosides was refluxed 1 hr with 1 N potassium hydroxide. The mixture was then acidified with hydrochloric acid (to 1 N) and extracted repeatedly with chloroform. The combined chloroform extracts were distilled to remove most of the solvent; the residue was steam-distilled. The steam distillate was extracted with ether, from which was obtained, after drying with sodium sulfate and evaporation, 0·34 g of liquid acidic material.

The residual aqueous liquor was extracted repeatedly with chloroform. The combined chloroform extracts, after drying with sodium sulfate and evaporation, yielded 2·05 g of deacylated and *degraded* glycosides whose i.r. spectrum (as a liquid film) had strong maxima at 2·91 μ, 3·41 μ, 5·78 μ, 9·35 μ, 13·22 μ and a moderate peak at 11·23 μ (β-glycoside).[26]

The acidic aqueous liquor remaining after chloroform extractions was neutralized with silver carbonate, filtered, and then treated with hydrogen sulfide to remove excess silver ion, filtered again, and evaporated to dryness *in vacuo*. An *acid-labile sugar fraction* (1·00 g) was thus obtained as a viscous syrup.

Characterization of the Steam-Volatile Acid

The acid fraction obtained by steam distillation was analyzed by gas–liquid chromatography on a Resoflex 446 column (¼ in.). A number of known acids were run under the same conditions. The retention times (min) observed for known acids were: acetic, 0·30; propionic, 1·03; isobutyric, 1·00; butyric, 1·40; isovaleric, 1·49; α-methylbutyric, 1·55; valeric, 2·05. The unknown acid mixture contained 3·5% of a component emerging in 1·20 min and 96·5% of one emerging in 1·60 min (a branched C_5-acid[42, 43]). A portion of this (0·30 g) was dissolved in 10 ml of dry, purified acetone; 1·0 g of potassium permanganate was added in small portions to the solution while heating at reflux.[19] After refluxing the mixture overnight, water was added and sufficient sodium bisulfite to reduce the manganese dioxide and permanganate. The resulting solution was acidified with hydrochloric acid and extracted repeatedly with ether. Combined ether extracts were dried over sodium sulfate and evaporated, leaving 0·069 g of degradation products. Gas–liquid chromatographic analysis of these products as described above indicated acetic acid to be the major acidic degradation product; no isobutyric acid was found. Thus the major volatile acid must be D- or L-α-methylbutyric acid.

Partial Degradation of Deacylated Glycoside by Acid Hydrolysis

An 0·42-g portion of deacylated glycoside was refluxed 6 hr with 1 N aqueous hydrochloric acid. The resulting mixture was then extracted several times with chloroform. Combined chloroform extracts, after drying with sodium sulfate and evaporation, yielded 0·30 g of

---

[41] E. L. JACKSON and C. S. HUDSON, *J. Amer. Chem. Soc.* **59**, 994 (1937).

[42] J. C. HAWKE, R. P. HANSEN and F. B. SHORLAND, *J. Chromatog.* **2**, 547 (1959).

[43] R. A. LANDOWNE and S. R. LIPSKY, *Biochem. et Biophys. Acta* **47**, 589 (1961).

degraded glycoside having an i.r. spectrum similar to that mentioned in the preceding section. Gas–liquid chromatographic analysis of the methyl ester of this product (prepared by diazomethane treatment) as well as its insolubility in CCl_4 indicated the absence of free aglycone (hydroxyhexadecanoate). The residual aqueous liquor, upon evaporation *in vacuo*, yielded 0·13 g of sugars (A and B).

A 3·0-g portion of degraded glycoside was suspended in 130 ml of 1 N hydrochloric acid for 2 hr. The product isolated by repeated extraction with chloroform was similar in its spectral and chromatographic properties to that described in the preceding paragraph. It yielded only Sugar C on more rigorous acid hydrolysis.

Acid Hydrolysis of Degraded Glycoside

(*a*) *With 1 N HCl/MeOH.*[16] Degraded glycoside (1·35 g) was refluxed 18 hr with 100 ml of methanol and 12·5 ml of conc. HCl. The mixture was then diluted with water and extracted repeatedly with chloroform. Combined chloroform extracts were dried with sodium sulfate and evaporated; 0·59 g of crude aglycon was obtained as a waxy methyl ester.

The residual aqueous liquor was neutralized with silver carbonate and filtered. The filtrate was treated with hydrogen sulfide, filtered, and evaporated to dryness *in vacuo*. The more acid-resistant sugar was obtained as a methyl glycoside, 0·94 g. An 0·050-g portion of this glycoside was refluxed overnight with 0·5 N aqueous HCl, then evaporated to dryness *in vacuo* after treatment with silver carbonate and hydrogen sulfide as previously described; 0·50 g of Sugar C was obtained as a syrup.

(*b*) *With 1 N HCl/50% dioxane.*[17] Degraded glycoside (2·00 g) was refluxed 2 hr with 1 N HCl in 50% aqueous dioxane. The mixture was then extracted with petroleum ether (30–60°). Combined, dried petroleum ether extracts yielded upon evaporation 1·09 g of crystalline aglycon. The residual liquor was treated with silver carbonate and hydrogen sulfide as described previously and evaporated to dryness *in vacuo*. The acid-resistant sugar fraction (C) was obtained as a syrup (1·00 g).

Characterization of the Aglycon

(*a*) *As a methyl ester.* An 0·57-g portion of the crude methyl ester was recrystallized from hexane; 0·14 g was obtained, m.p. 41·5–44° (Ref. 6, m.p. methyl ester of jalapinolic acid, 46–47°); $[\alpha]_D^{26}+0{\cdot}8$ (*c* 5·4, EtOH); i.r. maximum (1% in CCl_4) at 2·68 μ (OH). Gas–liquid chromatographic analyses as described by Miwa *et al.*[36] indicated this ester was 97% pure monohydroxyhexadecanoate having a normal carbon skeleton.

Oxidative cleavage of the methyl ester (0·11 g) with chromium trioxide–acetic acid was carried out essentially as described by Meakins and Swindells.[44] At the completion of the oxidation, the reaction mixture was diluted with ice water and extracted with petroleum ether–ethyl ether (1:1). The combined extracts were, in turn, extracted with 5% potassium carbonate. The combined carbonate extracts were acidified with HCl and extracted repeatedly with ethyl ether. Combined ether extracts were dried with sodium sulfate and evaporated cautiously so as to retain volatile acids. These mixed cleavage products (0·13 g) were shown by gas–liquid chromatographic analyses (both before and after esterification with diazomethane) to be mainly C_5 and C_6 monocarboxylic acids together with C_{10} and C_{11} dicarboxylic acids (or esters)—all straight chain.

(*b*) *As a free acid.* A 1·05-g portion of crude aglycon was recrystallized from hexane; 0·75 g, m.p. 63–64°, was obtained. This was recrystallized from hexane–chloroform, then

[44] G. D. Meakins and R. Swindells, *J. Chem. Soc.* 1044 (1959).

from aqueous acetone; 0·45 g, m.p. 64–65°, was obtained (Ref. 6, m.p. for jalapinolic acid, 65·5–66·5°). (Found: C, 70·8; H, 11·7. Calc. for $C_{16}H_{32}O_3$: C, 70·5; H, 11·8.)

The residue obtained by evaporating the filtrate from the first crystallization (0·207 g) was esterified with 1% methanolic H_2SO_4. The ester obtained was subjected to gas chromatographic analysis. No hydroxy ester other than hydroxyhexadecanoate was found; small amounts of linoleate and palmitate appeared to be present.

Characterization of Acid-Labile Sugar Fraction (Sugars A and B)

The acid-labile sugar fraction was subjected to paper chromatography on Whatman No. 4 paper with the solvent system ethyl acetate–pyridine–water (120:50:40). The papers were sprayed with the silver nitrate/acetone reagent.[20, 45] Two well-separated spots (A and B) of nearly equal intensity were developed (Table 1). Solvent systems V and VII described by Krauss *et al.*[23] were also applied but gave less satisfactory resolution. Spraying spots of these sugars with the vanillin–perchloric acid reagent of MacLennan *et al.*[22] gave a light orange color that faded to gray and closely resembled spots of L-rhamnose and L-fucose developed simultaneously. Sugars A and B gave light olive colors when sprayed with *o*-aminobiphenyl as described by Timell *et al.*,[46] in contrast to pentoses, which gave a yellow–orange color.

TABLE 1. TYPICAL RESULTS OBTAINED IN PAPER CHROMATOGRAPHY OF SUGARS OBTAINED BY HYDROLYSIS OF *Ipomoea parasitica* GLYCOSIDES

Sugar	Solvent system† I	II	III
Known:	R_f	R_r*	R_r*
L-Fucose	0·69	0·81	0·77
6-Deoxy-D-gulose	0·76	0·95	0·86
L-Rhamnose	0·76	1·00	1·00
Unknown:			
A	0·68	0·78	0·74
B	0·75	0·91	0·87
C	0·74	0·93	0·88

† System I: EtOAc–pyridine–H_2O (120:50:40).[20]
System II: methylethyl ketone–H_2O (system V of Krauss *et al.*[23]).
System III: methylethyl ketone–*n*-butanol (1:1)/H_3BO_3-$Na_2B_4O_7$ (system VII of Krauss *et al.*[23]).
* R_r = rate relative to rhamnose.

An 0·33-g portion of the crude syrup was treated with conc. HCl and ethyl mercaptan essentially as described by Zinner.[47] A crude yield of 0·15 g of mixed diethyl dithioacetals, m.p. 145–146°, was obtained. Two different derivatives were obtained when this mixture was recrystallized from water. The *first crop*, 0·033 g derived from Sugar A, had m.p. 166–167°; after recrystallization from chloroform it gave 0·021 g, m.p. 166·5–167°. On admixture with authentic L-fucose diethyl dithioacetal (m.p. 166–167°; lit.[48] m.p. 166–167°), the m.p. was

---

[45] W. E. TREVELYAN, D. P. PROCTER and J. S. HARRIS, *Nature, Lond.* **166**, 444 (1950).
[46] T. E. TIMELL, C. P. J. GAUDEMANS and A. L. CURIE, *Anal. Chem.* **28**, 1916 (1956).
[47] H. ZINNER, *Chem. Ber.* **84**, 780 (1951).
[48] L. H. STERNBACH, S. KAISER and M. W. GOLDBERG, *J. Am. Chem. Soc.* **80**, 1639 (1958).

depressed to 158–163°. The two substances had identical X-ray diffraction patterns however and thus must be optical antipodes.*

The *second crop* from the above crystallization mixture, 0·015 g derived from Sugar B, had m.p. 147–147·5° and was recrystallized from chloroform–hexane without observable change in melting point. (Found: C, 44·6; H, 8·3. $C_{10}H_{22}O_4S_2$ requires: C, 44·4; H, 8·2.) No change in m.p. was observed when 0·5 mg of this derivative was crystallized from chloroform in the presence of a seed crystal of 6-deoxy-D-allose diethyl dithioacetal (m.p. 99–100°), or when the latter substance was similarly crystallized in the presence of a seed crystal of Sugar B diethyl dithioacetal.

Characterization of Acid-Resistant Sugar Fraction (Sugar C)

The acid-resistant sugar fraction was subjected to paper chromatography by the same methods used for Sugars A and B. A single spot was obtained in all cases. Results are summarized in Table 1. The Dische–Shettles color test[21] suggested that Sugar C was a 6-deoxyhexose; the color yield for Sugar C in the Disch–Shettles procedure was about 2·3 times that obtained for L-fucose. The vanillin–perchloric acid color test[22] also indicated a 6-deoxyhexose.

The diethyl dithioacetal of Sugar C (0·257 g) was prepared essentially by the method of Zinner.[47] The product would not crystallize from the reaction mixture. The aqueous acidic mixture was extracted repeatedly with chloroform. The combined chloroform extracts, after drying with sodium sulfate and evaporation, yielded 0·115 g, m.p. 92·5–94°. This product was recrystallized from chloroform–hexane; 0·075 g of derivative, m.p. 94–97°, was obtained. (Found: C, 44·8; H, 8·2; S, 23·6. Calc. for $C_{10}H_{22}O_4S_2$: C, 44·4; H, 8·2; S, 23·7.) The mixed m.p. with authentic 6-deoxy-L-glucose diethyl dithioacetal (m.p. 97·5–98·5°; Ref. 49, m.p. 97–98°) showed considerable depression. The two diethyl dithioacetals had identical X-ray diffraction patterns however and thus must be optical antipodes.*

The optical rotation of crude Sugar C was $[\alpha]_D^{23}$ +23° (c 6·7, H_2O) (lit.[50] value for 6-deoxy-D-glucose, +29·7°).

Diethyl Dithioacetal of 6-deoxy-D-allose

6-Deoxy-D-allose (5·6 mg) was stirred 10 min at 0° with 0·025 ml of conc. HCl and 0·01 ml of ethyl mercaptan. The reaction mixture was extracted several times with small portions of chloroform. The combined extracts were dried with sodium sulfate and evaporated; the yield was 3·5 mg of diethyl dithioacetal, m.p. 85–89°. Recrystallization from chloroform–petroleum ether raised the m.p. of this product to 99–100°. Because of the smallness of the sample, elementary analyses could not be obtained.

Acknowledgements—We are indebted to Professor T. Reichstein, University of Basel, and Dr. N. K. Richtmeyer, National Institutes of Health, for gifts of sugar specimens, and to Dr. Q. Jones, New Crops Research Branch, Agricultural Research Service, U.S. Dept. of Agriculture, for providing *Ipomoea* seeds and for identifying them botanically. We also thank Mr. J. Hodge, Dr. J. H. Sloneker and Dr. H. L. Tookey for helpful suggestions and discussions; Messrs. J. W. Hageman and K. L. Mikolajczak for gas chromatographic analyses; Mrs. Clara McGrew for microanalyses; and Mr. Marvin Bagby for carrying out portions of the experimental work.

* The rings obtained in the X-ray diffraction patterns of the two compounds are identical, both in regard to spacing and intensity. From the basic concepts of X-ray crystallography it therefore follows that the two compounds must either be identical or have a mirror-image relationship. [Cf. S. C. Nyburg, *X-ray Analaysis of Organic Structures*, p. 134, Academic Press, New York (1961).] It would be expected that optical antipodes *would depress* each other's melting points [cf. R. L. Shriner, R. Adams and C. S. Marvel in *Organic Chemistry*, 2nd Edition (Edited by H. Gilman), Vol. 1, p. 248, John Wiley, New York (1943)].

[49] E. Zissis, N. K. Richtmeyer and C. S. Hudson, *J. Amer. Chem. Soc.* **73**, 4714 (1951).

[50] W. Pigman, *The Carbohydrates*, p. 80, Academic Press, New York (1957).