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A New Xanthone C-Glucoside, Position Isomer of Mangiferin, from *Anemarrhena asphodeloides* Bunge[1]

Masakazu Aritomi[2a] and Toshio Kawasaki[2b]

Faculty of Education, Kumamoto University[2a] and Faculty of Pharmaceutical Sciences, Kyushu University[2b]

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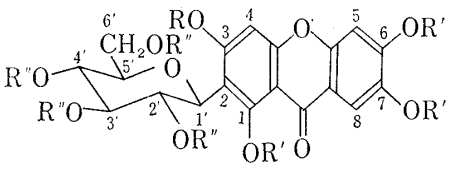
A new xanthone C-glucoside, $C_{19}H_{18}O_{11}\cdot\frac{1}{2}H_2O$, decomposing over 260° without melting, $[\alpha]_D^{21}$ +5.5° (pyridine), was isolated in a pure state along with mangiferin (I) from the aerial parts of *Anemarrhena asphodeloides* Bunge (Liliaceae) and named isomangiferin. On the basis of chemical and spectral data, it has been assigned the structure 4-C-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone (XV), the first position isomer of I to be found and isolated. The relation between XV and I, in structures and properties, is corresponding to that between flavonoid 8-C- and 6-C-glucosides such as orientin and isoorientin.

Mangiferin (I), the structure of which has recently been established as 2-C-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone,[3] was first isolated from *Mangifera indica* L.[4] (Anacardiaceae), and later from several different families of plants.[5] In spite of the fact that it is rather widely distributed and a number of free xanthone derivatives and O-glycosides are also known to occur in plants,[5] I has been the only natural xanthone C-glycoside until the recent isolation[6] of its 3-monomethyl ether, homomangiferin (II).

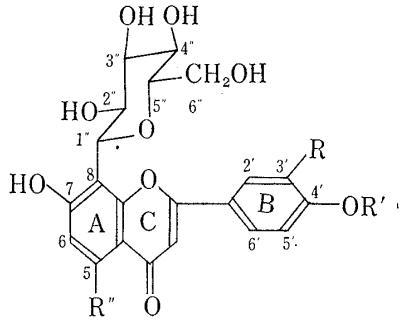
As for the seemingly related flavonoid C-glycosides, several kinds of natural compounds appear in the literatures and there are some pairs of coexisting position isomers, 8-C- and 6-C-glucosides, such as vitexin (III) and isovitexin (IV),[7] orientin (V) and isoorientin (VI),[7,8] 8-C- (VII) and 6-C- (VIII) β-D-glucopyranosyldiosmetins,[9] and isohemiphloin (IX) and hemiphloin (X).[10] Their formation in plants had been assumed[11] to involve the conjugation of glucosyl residue to an anionoid center of aromatic ring of flavonoid nucleus at the final step

1) Preliminary communication: M. Aritomi and T. Kawasaki, *Tetrahedron Letters*, **1969**, 941.
2) Location: *a*) *Kurokami-machi, Kumamoto*; *b*) *Katakasu, Fukuoka.*
3) *a*) D. Billet, J. Massicot, C. Mercier, D. Anker, A. Matschenko, C. Mentzer, M. Chaigneau, G. Valdener and H. Pacheco, *Bull. Soc. Chim. France*, **1965**, 3006; *b*) L.J. Haynes and D.R. Taylor, *J. Chem. Soc.* (*C*), **1966**, 1685; *c*) V.K. Bhatia, J.D. Ramanathan and T.R. Seshadri, *Tetrahedron*, **23**, 1363 (1967); *d*) P.E. Nott and J.C. Roberts, *Phytochemistry*, **6**, 741 (1967); *e*) *Idem, ibid.*, **6**, 1597 (1967); *f*) V.K. Bhatia and T.R. Seshadri, *Tetrahedron Letters*, **1968**, 1741.
4) W. Wiechowski, *Lotos*, **56**, 61 (1908) (cited from A.L. van Scherpenberg, *Rec. Trav. Chim.*, **35**, 346 (1916) [*C.A.*, **11**, 41 (1917)]).
5) *a*) I. Carpenter, H.D. Locksley and F. Scheinmann, *Phytochemistry*, **8**, 2013 (1969); *b*) T. Tomimori and M. Komatsu, *Yakugaku Zasshi*, **89**, 410 (1969); *c*) *Idem, ibid.*, **89**, 1276 (1969).
6) M. Aritomi and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 760 (1968); *idem, ibid.*, **18**, 2224 (1970).
7) *a*) M. Aritomi, *Yakugaku Zasshi*, **83**, 737 (1963); *b*) H. Wagner, J. Patel, L. Horhammer, F. Yap and A. Reichardt, *Zeitschrift für Naturforsch.*, **22b**, 988 (1967); *c*) M.K. Seikel, D.J. Hölder and R. Birzgalis, *Arch. Biochem. Biophys.*, **85**, 272 (1959).
8) *a*) B.H. Koeppen, C.J.B. Smit and D.G. Roux, *Biochem. J.*, **83**, 507 (1962); *b*) M. Yasue, M. Itaya, H. Oshima and S. Funahashi, *Yakugaku Zasshi*, **85**, 553 (1965); *c*) M.K. Seikel and A.J. Bushnell, *J. Org. Chem.*, **24**, 1995 (1959).
9) B. Gentili and R.M. Horowitz, *J. Org. Chem.*, **33**, 1571 (1968).
10) W.E. Hillis and D.H.S. Horn, *Australian J. Chem.*, **18**, 531 (1965).
11) *a*) W.B. Whalley, "Recent Development in the Chemistry of Natural Phenolic Compounds," ed. by W.D. Ollis, Pergamon Press, Oxford, London, New York, Paris, 1961, p. 20; *b*) L. Hörhammer and H. Wagner, *ibid.*, p. 185.

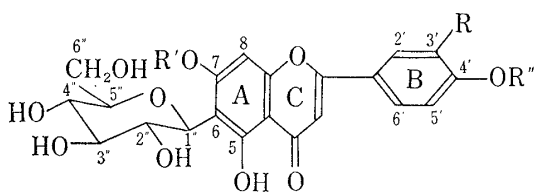
of biosynthesis, but lately Wallace, *et al.*[12] reported that the C-glucosylation takes place at the chalcone stage prior to the ring closure to form flavonoid nucleus. In view of the generally accepted biosynthetic scheme[13] of flavonoids, the coexistence of 8-C- and 6-C-glucosylflavonoids as mentioned above and the cooccurrence of dihydrochalcone C-glucoside, aspalathin (XI),[8a,14] and flavone C-glucosides, V as well as VI, in *Aspalathus linearis*[14] are better accounted for by the Wallace's presumption. The natural xanthones in higher plants are thought to be biosynthesized by intramolecular ring closure[5a] of a benzophenone-like intermidiate which is formed by condensation of a β-triketide with a unit originated from shikimate.[5a] Maclurin (XII) has been proposed as the precursor of 1,3,6,7-tetrahydroxyxanthone (XIII),[5a,15] the aglycone of I, on the basis of the facts that XII is converted to XIII by irradiation of ultraviolet (UV) light[15a] and by oxidative coupling *in vitro*[15b] and that XII exists together with XIII and its isomer, 1,3,5,6-tetrahydroxyxanthone (XIV) in *Symphonia globuifera* L.[15c] Nott, *et al.*[3e] and Bhatia, *et al.*[3f] have reported that I might be formed in plant *via* C-glucosylation of XIII, but, by analogy with the flavonoid C-glucosides, it seems rather probable that not XIII but XII is C-glucosylated and subsequently converted to I. If it is assumed that sugar conjugation takes place only on the phloroglucinol moiety of XII because all known flavonoid C-glucosides have the sugar moiety on A ring, the resulting 3- (or 5-) C-glucosylmaclurin could yield four isomeric xanthone derivatives, 2-C- and 4-C-glucosides of XIII and XIV (I, XV, XVI and XVII). Therefore coexistence of I with these three isomers and also with 3- (or 5-) C-glucosylmaclurin would not be improbable.



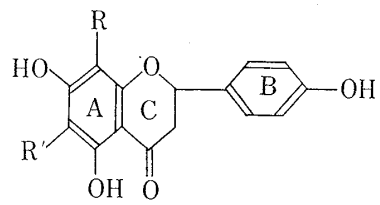
I : R=R′=R″=H
II : R=Me, R′=R″=H
XXI : R=R′=Me, R″=H
XXIII : R=R′=R″=Me
XXV : R=R′=Me, R″=Ac



III : R=R′=H, R″=OH
V : R=R″=OH, R′=H
VII : R=R″=OH, R′=Me
XXVI : R=R′=R″=H

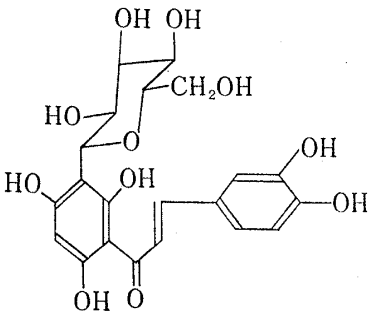


IV : R=R′=R″=H
VI : R=OH, R′=R″=H
VIII : R=OH, R′=H, R″=Me

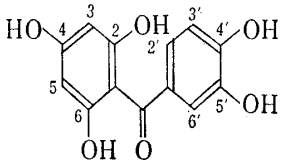


IX : R=β-D-glucopyranosyl, R′=H
X : R′=β-D-glucopyranosyl, R=H

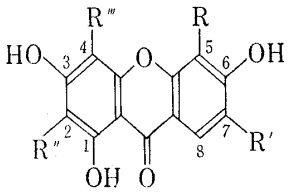
12) J.W. Wallace and R.E. Alston, *Plant and Cell Physiol.*, **7**, 699 (1966).
13) *a*) T.A. Geissman, "Biogenesis of Natural Compounds," ed. by P. Bernfeld, Pergamon Press, Oxford, London, New York, Paris, 1963, p. 563; *b*) H. Grisebach, "Biosynthetic Patterns in Microorganisms and Higher Plant," John Willey and Sons Inc., New York, London, Sydney, 1967, p. 1; *c*) E. Wong, *Phytochemistry*, **7**, 1751 (1968).
14) B.H. Koeppen and D.G. Roux, *Tetrahedron Letters*, **1965**, 3497.
15) *a*) A. Jefferson and F. Scheinmann, *J. Chem. Soc.* (*C*), **1966**, 175; *b*) J.E. Atkinson and J.R. Lewis, *ibid.* (*C*), **1969**, 1386; *c*) H.D. Locksley, I. Moore and F. Scheinmann, *Tetrahedron*, **23**, 2229 (1967).



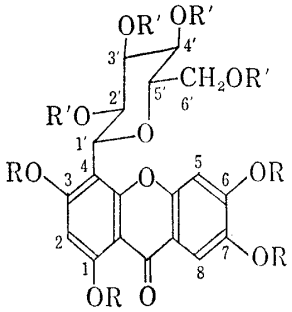
XI



XII



XIII : R=R″=R‴=H, R′=OH
XIV : R=OH, R′=R″=R‴=H
XVI : R=OH, R′=R‴=H, R″=β-D-glucosyl
XVII : R=OH, R′=R″=H R‴=β-D-glucosyl



XV : R=R′=H
XVIII : R=R′=Ac
XIX : R=Me, R′=H
XX : R=Me, R′=Ac
XXII : R=R′=Me

This paper concerns the isolation of one of the isomers, named isomangiferin and identified as 4-C-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone (XV)[16] as a minor constituent of the aerial parts of *Anemarrhena asphodeloides* Bunge (Liliaceae) in which the presence of I had been reported by Morita, *et al.*[17]

The extractives of the fresh material collected in Fukuoka during May revealed on paper chromatogram (PPC) two spots of phenolic compounds at *Rf* 0.50 and about 0.8, besides that (*Rf* 0.61) of I, and separation of the components was successively achieved as described in experimental part to give I (yield, 1.1%) and the compound of *Rf* 0.50 (XV) in 0.2% yield.

Chromatographically pure XV was obtained as yellow needles, $[\alpha]_D^{21}$ +5.5° (pyridine), which gradually decomposed over 260° without melting and analyzed for $C_{19}H_{18}O_{11}\cdot\frac{1}{2}H_2O$. Its UV spectrum, λ_{max}^{EtOH} mμ: 242, 257, 313 and 365, and the colors developed with magnesium-hydrochloric acid, zinc-hydrochloric acid, ferric chloride and sodium carbonate were all alike those[6] of I and II. XV resisted a conventional hydrolysis with hydrochloric acid, and on acetylation with acetic anhydride and sulfuric acid provided an acetate (XVIII), $C_{35}H_{34}O_{19}$, mp 203—204°, $[\alpha]_D^{20}$ −64.7° ($CHCl_3$), and on treatment with diazomethane gave a methyl ether (XIX), $C_{23}H_{26}O_{11}\cdot H_2O$, mp 292—293° (decomp.), $[\alpha]_D^{24}$ −13.2° (pyridine). XVIII showed in nuclear magnetic resonance (NMR) spectrum the signals due to four acetoxyl groups each on sugar and xanthone moieties and three singlets of aromatic protons (Table I), and XIX was negative to the ferric chloride test and yielded, on acetylation, an acetate (XX), $C_{31}H_{34}O_{15}$, mp 142—143°, $[\alpha]_D^{9}$ −9.8° ($CHCl_3$). The molecular formulae and the above properties of XV and its derivatives strongly suggest that XV is likely to be a xanthone C-glycoside similar to I and II. Refluxing of XV with hydroiodic acid in phenol followed by acetylation of the product[18] provided the tetraacetate of XIII, mp 197°, and on oxidation with ferric chloride according to the procedure of Hay, *et al.*[19] XV gave arabinose (major) and glucose.

16) Prof. V.G. Khrjanovski, All-Union Research Institute of Medical Plants, Moscow, informed us (Oct., 13, 1969) of his recent isolation of isomangiferin from a plant of Hedusarum genus.
17) N. Morita, M. Shimizu and M. Fukuta, *Yakugaku Zasshi*, **85**, 374 (1965).
18) S. Iseda, *Bull. Chem. Soc. Japan*, **30**, 625 (1957).
19) J.E. Hay and L.J. Haynes, *J. Chem. Soc.*, **1956**, 3141.

Since the Wessely–Moser rearrangement[20] of the xanthone moiety is not considered[21] to have taken place during the cleavage reaction with hydroiodic acid and the UV spectrum of XV, almost identical with those[6] of I, II and XIII, is quite different from the reported one[22] of XIV, XV is safely regarded as one of the C-glucosides of XIII. Oxidation of XV with potassium permanganate in alkaline medium[23] gave D-arabonic acid γ-lactone[24] which was also obtained on the same treatment of the reference compounds, I and 1-phenyl-1-deoxy-β-D-glucopyranose. Furthermore XIX consumed, in common with tetra-O-methylmangiferin (XXI) run in parallel, two moles of periodate without providing formaldehyde. Therefore it is evident that XV has a D-glucopyranose residue.

In the NMR spectrum of XVIII (Table I) the signals due to four acetoxyl groups each on xanthone (2.33, 2.35 and 2.43 ppm) and sugar (1.73, 2.03, 2.05 and 2.08 ppm) moieties[3a,b,d] are observed, and good agreement of the chemical shifts of the latter four with those of the corresponding functions of acetylated flavonoid C-β-D-glucopyranosides[10] and especially existence of the signal at 1.73 ppm strongly suggested[10] the C-β-D-glucosidic nature of XVIII. The NMR spectrum of permethyl ether of XV (XXII), mp 167°, prepared by Kuhn's exhaustive methylation[25] of XIX showed, when measured at 22° (Table I),[26] the signal of the anomeric

Table I. NMR Spectral Data of Derivatives of Isomangiferin and Mangiferin ($CDCl_3$, δ (ppm))

Temperature / Proton	Compound XVIII 23°	XX 23°	XXII 22°	XXII 60°	XXIII 22°
2-H	6.88(H, broad s)	6.38(H, s)	6.40(H, s)	6.43(H, s)	
4-H					6.64(H, s)
5-H	7.51(H, broad s)	6.93(H, s)	6.83(H, broad s)	6.83(H, s)	6.80(H, s)
8-H	8.00(H, s)	7.63(H, s)	7.63(H, s)	7.65(H, s)	7.61(H, s)
1′-H			about 5 (H)	4.97(H, d, J=10 cps)	4.81(H, d, J=10 cps)
OAc and OMe On xanthone Nucleus	2.33(3H, s) 2.35(3H, s) 2.43(6H, s)	3.99 4.03 4.09 } (12H, 3s)	3.97(6H, s) 4.02(6H, s)	3.96(6H, s) 4.01(6H, s)	3.92 3.94 } (3H); 3.96 3.98 4.04(sh) } (9H, 3s)
OAc and OMe On sugar Moiety	1.73 1.80(sh) } (3H); 2.03 2.05 2.08 } (9H, 3s)	1.66(3H, s) 2.05 2.08 2.10 } (9H, 3s)	3.13 3.17 } (3H); 3.39(3H, s) 3.65(3H, s) 3.72(3H, s)	3.14(3H, s) 3.37(3H, s) 3.63(3H, s) 3.71(3H, s)	3.17(3H, s) 3.35(3H, s) 3.59(3H, s) 3.66 3.69 } (3H)

s: singlet, d: doublet, sh: shoulder

20) F. Wessely and G.H. Moser, *Monatsh. Chem.*, **56**, 97 (1930).
21) It requires much more drastic condition (E.M. Philbin, J. Swirski and T.S. Wheeler, *J. Chem. Soc.*, **1956**, 4455).
22) M.L. Wolfrom, F. Komitsky, Jr. and P.M. Mundell, *J. Org. Chem.*, **30**, 1088 (1965).
23) K. Funaoka, *Journal of the Japanese Wood Research Society*, **3**, 218 (1957).
24) Identified with a sample prepared after the procedure of Jensen, *et al.* (F.W. Jensen and F.W. Upson, *J. Am. Chem. Soc.*, **47**, 3019 (1925)).
25) R. Kuhn, H. Trischmann and I. Löw, *Angew. Chem.*, **67**, 32 (1955).
26) An anomalous splitting of a methoxyl signal at 22° but not at 60°, which had been encountered in the spectra of II and its heptaacetate in pyridine solution (20° and 50°),[6] was also observed in the spectrum of XXII (Table I). The spectrum of permethyl ether of I (XXV) at 22° showed a similar anomaly in the methoxyl signals (Table I).

proton of sugar residue as a set of weak peaks around at 5 ppm and three aromatic protons as two sharp (6.40 and 7.63 ppm) and one broad singlets (6.83 ppm),[27] while the spectrum[26] measured at 60° (Table I) showed the anomeric proton as doublet (J=10 cps) centering at 4.97 ppm and the three protons of xanthone nucleus as three sharp singlets (7.65, 6.83 and 6.43 ppm).[27]

The above spectral data indicate the β-linkage of the D-glucopyranose residue in C-1 conformation and absence of one of the *meta*-located aromatic protons at C-2 and C-4. The chemical shifts (7.65 and 6.83 ppm, at 60°) of two aromatic protons of XXII are same as those of permethyl ether of I (XXIII) at 7.61 and 6.80 ppm, which are assigned[3a,b,d] respectively to the protons at C-8 and C-5. The singlet at 6.43 ppm of XXII appears at a significantly higher field than that (6.64 ppm) of the C-4 proton of XXIII, and in consideration of the reported observation[3a,b,d] that the signal of C-2 proton in many derivatives of XIII is always found at the higher field than that at C-4 proton, it could be ascribed to the proton at C-2, and hence the C-glucosidic linkage in XXII is to be located at C-4. An additional support for the site of sugar conjugation was furnished by the fact that XV was negative to the Gibbs reagent[28] while I and II were positive.

Consequently XV is assigned the structure 4-C-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone.

As reported in a separate paper,[29] mass spectral difference[30] between XX and acetate of XXI (XXV) is similar to that between methyl ether acetates of III and IV, which hold the same partial structure with XV and I, respectively.

Rf value (0.50) on PPC and optical rotation ($[\alpha]_D^{21}$ +5.5° (pyridine)) of XV are significantly smaller than those[6] (*Rf* 0.61, $[\alpha]_D^{16}$ +35.5° (pyridine)) of I, and these facts are also consistent with the empirical rules on flavonoid 8-C- and 6-C-glucosides.[10,31]

From the biogenetic view point it is of interest that XV is first isolated together with I and the relation between them is corresponding to that between V and VI.

Experimental[32]

Isolation of Isomangiferin (XV) from the Aerial Parts of *A. asphodeloides* BUNGE——The fresh material (300 g) collected during May in the garden of Kyushu University was extracted three times with refluxing MeOH. The extracts (PPC: *Rf* 0.50, 0.61 (major), 0.8 (trace)) were combined, concentrated, filtered and

27) Eade, *et al.* (R.A. Eade, W.E. Hillis, D.H.S. Horn and J.J. Simes, *Australian J. Chem.*, **18**, 715 (1965)) reported that an aromatic proton of the acetates of vitexin (III) and bayin (XXIV) appears in the NMR spectrum taken at room temperature as a doublet or a broad singlet, but, at the higher temperature, it is detected as a sharp singlet.

28) H.D. Gibbs, *J. Biol. Chem.*, **72**, 649 (1927).

29) M. Aritomi, T. Komori and T. Kawasaki, *Ann. Chem.*, **734**, 91 (1970).

30) In the preliminary communication[1] it was reported that the mass spectra of free XV and I failed to give any particular information concerning their standard differences but it should be amended as described in a separate paper.[29] The authors are grateful to Dr. A. Prox, Organisch-chemischen Institut, Technischen Hochschule, München, for the kind advice.

31) E.C. Bate-Smith and T. Swain, *Chem. Ind.*, **1960**, 1132.

32) Melting points were measured on a Kofler block unless otherwise stated and are uncorrected. Optical rotations were taken with a JASCO automatic polarimeter Model DIP-SL and NMR spectra were determined on a JEOL-JNM-C-60H spectrometer at 60 MHz using Me_4Si as internal reference. Chemical shifts are given in δ (ppm) values. Mass spectra were determined at an ionizing voltage of 40 eV with a JMS-01SG mass spectrometer. UV spectra were taken on a Shimazu SV-50A automatic spectrometer and IR spectra were recorded on a JASCO DS-301. PPC of phenolic compounds were run on Toyo Roshi No. 50 in ascending method using 30% AcOH as a solvent and the spots were detected by spraying with 5% Na_2CO_3 solution. For TLC, "Kieselgel G nach Stahl" was used and for column chromatography, Kanto silica gel (100—200 mesh) was employed. Polyamide powder (M=8000. Particle size: <15 μ, 9%; 15—20 μ, 14%; 20—30 μ, 31%; 30—40 μ, 18%; 40—95 μ, 15%; >95 μ, 13%) was kindly donated by Ube Industries Ltd.

the filtrate was left stand overnight. The precipitates were collected by filtration, washed with MeOH and crystallized from dioxane–H_2O (1:1, v/v) to give pale yellow needles (2.4 g), $[\alpha]_D^{28}$ +37.6° (c=0.85, pyridine), which decomposed over 250° without melting and showed the same infrared (IR) spectrum and *Rf* value (0.61) on PPC as those of authentic I obtained from *Mangifera indica* L.[6] The mother liquor was extracted with $CHCl_3$ (4 times) and then with EtOAc(20 times). The EtOAc extractive, which revealed on PPC three spots of phenolic compounds at *Rf* 0.50, 0.61 (main) and about 0.8, were digested with MeOH (about 15 ml) to yield further crop of I (0.8 g). The filtrate was passed through a column of polyamide powder (30 g) and the column was washed with H_2O and then percolated with MeOH. The MeOH percolate was concentrated and left stand for several days to give a crystalline mass (600 mg), which showed two spots on PPC, *Rf* 0.50 and 0.61 (trace). It was refluxed with MeOH (about 20 ml) and the hardly soluble portion was collected to give chromatographically homogeneous compound of *Rf* 0.50 (500 mg). Its crystallization from dioxane–H_2O (1:1, v/v) gave XV as yellow needles which gradually decomposed over 260° without melting. $[\alpha]_D^{21}$ +5.5° (c=1.00, pyridine). *Anal.* Calcd. for $C_{19}H_{18}O_{11}\cdot\frac{1}{2}H_2O$: C, 52.91; H, 4.44. Found: C, 52.76; H, 4.36. UV λ_{max}^{EtOH} mμ (log ε): 242 (4.44), 257 (4.58), 313 (4.28), 365 (4.10). It gave the same colors with Mg- and Zn-HCl (orange) and with $FeCl_3$ (dark green) as I and II, and showed on PPC a spot which revealed brown color with yellow fluorescence by spraying with 5% Na_2CO_3 solution. It is sparingly soluble in many organic solvents except pyridine and dioxane, and soluble with yellow coloration in Na_2CO_3 and NaOH solutions and in warm conc. HCl. The solution (yellow) of XV in borate buffer (pH 9) gave no significant color change with Gibbs reagent[28] but those (yellow) of I and II turn to intense green.

Treatment of XV with HCl——A mixture of XV (20 mg) and conc. HCl (2 ml) was heated on a water bath for 10 min and diluted with H_2O. The precipitates were collected by centrifugation, washed with H_2O and examined by PPC. Only one spot of phenolic compound was detected at *Rf* 0.50, identical with that of XV run in parallel. The supernatant was neutralized by passing through a column of Amberlite IR 45 and concentrated *in vacuo* to leave a trace amount of syrup, which was negative to Molisch reaction and gave no spot of reducing sugar on PPC.

Isomangiferin Octaacetate (XVIII)——XV (100 mg) was acetylated with Ac_2O (5 ml) and one drop of conc. H_2SO_4 at room temperature overnight. The reaction mixture was poured into ice–water and the precipitates were collected, washed with H_2O, dried and crystallized from $CHCl_3$–hexane (1:4, v/v) to yield XVIII as colorless needles, mp 203—204°, $[\alpha]_D^{22}$ −64.7° (c=1.12, $CHCl_3$). *Anal.* Calcd. for $C_{35}H_{34}O_{19}$: C, 55.41; H, 4.52. Found: C, 55.19; H, 4.53. NMR: Table I. It gave no color with $FeCl_3$.

Tetra-O-methylisomangiferin (XIX)——To a solution of XV (200 mg) in dimethylformamide (5 ml) and MeOH (40 ml) was added an ethereal solution of CH_2N_2 prepared from 4 g of nitrosomethylurea. After leaving stand at room temperature overnight, the solvent was removed (finally *in vacuo*) and the residue was triturated with a small amount of MeOH (1—2 ml) to give a crystalline product (130 mg), which revealed three spots[33] (the most polar one being major) on thin-layer chromatography (TLC) ($CHCl_3$–MeOH–H_2O (7:3:1, v/v, lower layer)). The mixture was subjected to column chromatography on silica gel (40 g) using $CHCl_3$–EtOH (10:1, v/v) as solvent, and the fractions containing only the most polar compound were combined and evaporated. The residue (100 mg) was crystallized from MeOH to give XIX as colorless needles, mp 292—293° (decomp.), $[\alpha]_D^{24}$ −13.2° (c=0.87, pyridine). *Anal.* Calcd. for $C_{23}H_{26}O_{11}\cdot H_2O$: C, 55.64; H, 5.68. Found: C, 55.61; H, 5.72. It gave no color with $FeCl_3$ and Na_2CO_3. UV λ_{max}^{EtOH} mμ (log ε): 254 (4.63), 355 (4.10).

Tetra-O-methylisomangiferin Tetraacetate (XX)——XIX was acetylated with Ac_2O and pyridine and worked up in the usual way. Recrystallization of the product from $CHCl_3$–hexane (1:4, v/v) afforded XX as colorless needles, mp 142—143°, $[\alpha]_D^{9}$ −9.8° (c=1.17, $CHCl_3$). Mol wt. Calcd. for $C_{31}H_{34}O_{15}$: 646.190. Found: 646.187 (mass spectrum). NMR: Table I.

Treatment of XV with HI——A solution of XV (0.3 g) in phenol (2.0 g) was refluxed with HI (*d*, 1.7, 3.0 ml) for 4 hr. The reaction mixture was poured into 5% $NaHSO_3$ solution (50 ml), and the precipitates were collected, washed with H_2O, dried and heated with Ac_2O and AcONa for 1 hr. The product was precipitated by adding H_2O, collected on filter paper, washed with H_2O and crystallized from EtOH to give XIII tetraacetate as colorless needles, mp 197°, undepressed on admixture with authentic sample[6] prepared from I in the same manner.

Oxidation of XV with $FeCl_3$——A suspension of XV (100 mg) in H_2O (15 ml) was refluxed with $FeCl_3\cdot 6H_2O$ (500 mg) for 6 hr. After being cooled, a black resin was filtered off and the brown filtrate was passed through columns of Amberlite IR 120 and 45. The resulting neutral solution was evaporated *in vacuo* to a syrup which was subjected to PPC by double ascending method using BuOH–pyridine–H_2O (3:2:1, v/v) as a solvent and *p*-anisidine·HCl as a staining reagent. The chromatogram showed two spots of reducing sugars and the running distances and colors of the major spot (13.3 cm, reddish brown) and the minor (11.9 cm, yellowish brown) were identical respectively with those of L-arabinose and D-glucose run in parallel.

33) Partial methylation of the sugar moiety of XV might have taken palce to give less polar products (M. Aritomi and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **18**, 677 (1970)).

Oxidation of XV with $KMnO_4$——To a solution of XV (800 mg) in 5% KOH solution (16 ml) was added a solution of $KMnO_4$ (2.4 g in 80 ml H_2O) during 3 hr under ice-cooling and stirring. MnO_2 was filtered off and the filtrate was neutralized by passing through a column of Amberlite IR 120 and evaporated *in vacuo* to give brown residue which was repeatedly extracted with hot EtOAc. The extract was concentrated to about 30 ml, diluted with benzene, filtered and the filtrate was gradually evaporated at room temperature to yield colorless needles. They were recrystallized from EtOAc–benzene (1:2, v/v) to give colorless needles, mp 92—93° (in capillary, H_2SO_4-bath), $[\alpha]_D^9$ +69.7° (c=0.71, H_2O). No depression of melting point was observed on admixture with authentic D-arabonic acid γ-lactone,[24] mp 95—96°. Treatment of I and 1-phenyl-1-deoxy-β-D-glucopyranose (syrup)[34] in the same way as above yielded the same product, mp 94—95° and 95—96°, respectively.

Oxidation of XIX with HIO_4——To a solution of XIX (27.8 mg, 5.8×10^{-5} mole) in a mixture of MeOH (10 ml) and H_2O (5 ml) was added a solution of $NaIO_4$ (400 mg in 40 ml H_2O) (5 ml), and the reaction mixture was diluted up to 25 ml with H_2O and kept in a dark. At intervals stated below an aliquot (5 ml) was taken for titration. The same reaction with a reference compound XXI and a blank test was carried out in parallel. Moles of NaI_4O consumed: 2 hr, 1.3; 4 hr, 1.4; 8 hr, 2.1; 24 hr, 2.2 (XXI: 2 hr, 1.2; 4 hr, 1.5; 8 hr, 1.9; 24 hr, 2.0).

The steam distillate of the reaction mixture gave, in either case of XIX or XXI, no color with chromotropic acid and conc. H_2SO_4, while in the case of D-glucose intense color (violet) appeared.

Permethyl Ether of XV (XXII)——A mixture of XIX (150 mg), CH_3I (2.0 g) and Ag_2O (2.0 g) was stirred in dimethylformamide for about 24 hr until the reaction mixture gave one spot (blue fluorescence) on TLC (benzene–MeOH (95:5, v/v)). The solids were filtered off, the filtrate was evaporated *in vacuo* and the residue was crystallized from dil. MeOH to give XXI as colorless needles (70 mg), mp 167°. Its IR spectrum gave no absorptions of OH group. NMR: Table I.

Permethyl Ether of I (XXIII)——XXI was treated in the same manner as above and yielded colorless needles (from dil. MeOH), mp 173°. NMR: Table I.

34) Prepared by deacetylation of 1-phenyl-1-deoxy-β-D-glucopyranose tetraacetate (C.D. Hurd and W.A. Bonner, *J. Am. Chem. Soc.*, **67**, 1972 (1945)) with NH_4OH in MeOH.