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Dehydro-*para*-asebotin, a New Chalcone Glucoside in the Flowers of *Gnaphalium affine* D. Don

Masakazu Aritomi[1a] and Toshio Kawasaki[1b]

Faculty of Education, Kumamoto University[1a] and Faculty of Pharmaceutical Sciences, Kyushu University[1b]

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Besides luteolin 4'-D-glucoside (I) and luteolin (II), a chalcone glycoside (III), $C_{22}H_{24}O_{10}\cdot 1\frac{1}{2}H_2O$, mp 199—200°, $[\alpha]_D^{30}$ −85.2° (methanol), ultraviolet spectrum (UV) λ_{max}^{EtOH} 368 nm, was isolated from the flowers of *Gnaphalium affine* D.Don. III was acid hydrolyzed to give glucose and a flavanone (V), mp 256°, UV λ_{max}^{EtOH} 228, 284 nm, which was identified as 4',7-dihydroxy-5-methoxyflavanone by chemical and spectral data and by direct comparison of the diethyl ether (VIII) with an authentic sample. The sample, 4',7-diethoxy-5-methoxyflavanone (VIII) was prepared, according to the result of a preliminary experiment (Table I), by stepwise alkylations of naringenin (IX) with an ether solution of diazoethane in absolute methanol for 3 hr and then with diazomethane in 70% methanol for 24 hr. Further examination of the methanolysis products of III permethylate (XV) and the spectral data of XV evidenced that III is 4'-O-β-D-glucopyranoside of 2',4,4'-trihydroxy-6'-methoxychalcone (dehydro-*para*-asebotin), from which V is yielded on acid treatment. This is the first isolation of III and V from natural sources.

In the previous paper,[2] the presence of luteolin 4'-D-glucoside (I) in the flowers of *Gnaphalium affine* D. Don was demonstrated. Now, in addition to I, luteolin (II) and a new chalcone glucoside (III) have been isolated from the same source, and this paper describes the identification of III as 2',4,4'-trihydroxy-6'-methoxychalcone 4'-β-D-glucopyranoside.

The methanol extracts of the fresh flowers were treated with lead acetate, and from the precipitates II was obtained, while the non-precipitable fraction gave I and III by polyamide column chromatography.

III was crystallized from dilute methanol as yellow needles, $C_{22}H_{24}O_{10}\cdot 1$ 1/2 H_2O, mp 199—200°, $[\alpha]_D^{30}$−85.2° (methanol), ultraviolet spectrum (UV) λ_{max}^{EtOH} 368 nm. It gave, on acetylation with acetic anhydride and pyridine, a syrupy acetate (IV), UV λ_{max}^{EtOH} 306 nm, and, on acid hydrolysis, an aglycone (V), $C_{16}H_{14}O_5$, mp 256°, UV λ_{max}^{EtOH} 228, 284 nm, and glucose in respective yields 59—66% and 37% (by anthrone method[3]).

V afforded a diacetate (VI), $C_{20}H_{18}O_7$, mp 174—175°, UV λ_{max}^{EtOH} 285 nm, a dimethyl ether (VII), $C_{18}H_{18}O_5$, mp 121°, UV λ_{max}^{EtOH} 228, 282 nm, and a diethyl ether (VIII), $C_{20}H_{22}O_5$, mp 121—122°, UV λ_{max}^{EtOH} 229, 282 nm, and was presumed to be 4',7-dihydroxy-5-methoxyflavanone on the basis of color reactions,[4] nuclear magnetic resonance (NMR)[5,6] and mass[7] spectral data and identification of VII with an authentic 4',5,7-trimethoxyflavanone.[8]

1) Location: *a*) *Kurokami, Kumamoto*; *b*) *Katakasu, Fukuoka.*
2) M. Aritomi, M. Shimojo, and T. Mazaki, *Yakugaku Zasshi*, **84**, 895 (1964).
3) K. Anno and N. Seno, "Seibutsu Kagaku Jikkenho," Vol. 11, Kyoritsu Shuppan Kabushiki Gaisha, Tokyo, 1968, p. 13.
4) K. Venkataraman, "The Chemistry of Flavonoid Compounds," ed. by T.A. Geissman, Pergamon Press, Oxford, 1962, p. 70.
5) T.J. Batterham and R.J. Highet, *Aust. J. Chem.*, **17**, 428 (1964).
6) T.J. Mabry, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, Berlin, Heidelberg, New York, 1970, p. 252.
7) S. Sasaki, Y. Itagaki, T. Kurokawa, E. Watanabe, and T. Aoyama, *Shitsuryo Bunseki*, **14**, 82 (1966).
8) K. Nakazawa and S. Matsuura, *Yakugaku Zasshi*, **75**, 469 (1955).

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In order to get the chemical evidence for the above presumption, it was hoped to prepare 4′,7-diethoxy-5-methoxyflavanone as a reference compound. So far, it has been reported that methylations of 4′,5,7-trihydroxyflavanone (naringenin) (IX) with an excess and an equimolar amount of diazomethane provide 4′,7-diether and 7-monoether, respectively.[9] As a preliminary experiment, methylation of IX with an ether solution of diazomethane in some different conditions was then undertaken, and it was found that the kind of product was dependent on solvent and reaction time. Thus, the reaction for 3 hr in anhydrous dioxane solution gave mainly 7-monomethyl ether (X), in anhydrous methanol for 3 hr provided 4′,7-dimethyl ether (XI) as an only isolable product, whereas in the reaction for 24 hr in 70% methanol even the chelated hydroxyl group was methylated to give 4′,5,7-trimethyl ether (VII) (Table I).

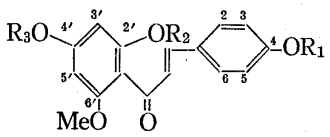
Table I. Yields (%) of Methyl Ethers on Methylation of Naringenin (IX) with Diazomethane–ether in Different Media and in Different Times

Products	Solvent and reaction time					
	Abs. dioxane		Abs. MeOH		70% MeOH	
	3 hr	24 hr	3 hr	24 hr	3 hr	24 hr
7-Mono-O-methylnaringenin (X)	70	47	+	−	−	−
4′,7-Di-O-methylnaringenin (XI)	0.5	5	42	33	22	−
Tri-O-methylnaringenin (VII)	−	−	+	31	48	67

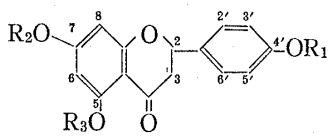
+: detected on TLC as a minor product but could not be isolated in crystalline state
−: could not be detected on TLC

Taking the above result into account, an analogous ethylation of IX in anhydrous methanol with an ether solution of diazoethane[10] for 3 hr was conducted and 4′,7-di-O-ethylnaringenin (XII), $C_{19}H_{20}O_5$, mp 97—98°, UV λ_{max}^{EtOH} 226, 289 nm, was obtained, accompanied by 7-mono-O-ethylnaringenin (XIII), $C_{17}H_{16}O_5$, mp 130—131°, UV λ_{max}^{EtOH} 222, 288 nm. Their structures were determined by color reaction with ferric chloride[4] and NMR spectral data[5,6] of XII and NMR[5,6] as well as mass[7] spectra of mono-O-methylated XIII (XIV), $C_{18}H_{18}O_5$, mp 111°, UV λ_{max}^{EtOH} 224, 289 nm. Subsequent methylation of XII in 70% methanol with diazomethane for 24 hr provided 4′,7-diethoxy-5-methoxyflavanone, $C_{20}H_{22}O_5$, mp 121—122°, which was identified with VIII, furnishing the definite proof for the structure V.

Treatment of III with dimethyl sulfate and potassium carbonate in dry acetone followed by the Kuhn's methylation[11] provided hexamethyl ether (XV), $C_{28}H_{36}O_{10}$, mp 112—113°,



III: $R_1=R_2=H$, $R_3=\beta$-D-glucopyranosyl
IV: $R_1=R_2=OAc$, R_3=tetra-O-acetyl-β-D-glucopyranosyl
XV: $R_1=R_2=Me$, R_3=tetra-O-methyl-β-D-glucopyranosyl
XVI: $R_1=R_2=Me$, $R_3=H$



V: $R_1=R_2=H$, $R_3=Me$
VI: $R_1=R_2=OAc$, $R_3=Me$
VII: $R_1=R_2=R_3=Me$
VIII: $R_1=R_2=Et$, $R_3=Me$
IX: $R_1=R_2=R_3=H$
X: $R_1=R_3=H$, $R_2=Me$
XI: $R_1=R_2=Me$, $R_3=H$
XII: $R_1=R_2=Et$, $R_3=H$
XIII: $R_1=R_3=H$, $R_2=Et$
XIV: $R_1=Me$, $R_2=Et$, $R_3=H$

9) J. Shinoda and S. Sato, *Yakugaku Zasshi*, **48**, 933 (1928).
10) K. Makino, A. Watanabe, and Y. Joh, *Seikagaku*, **32**, 788 (1960).
11) R. Kuhn, H. Trischmann, and I. Löw, *Angew. Chem.*, **67**, 32 (1955).

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$[\alpha]_D^{24}$ −18.6° (chloroform), UV λ_{max}^{EtOH} 327 nm, which was methanolyzed to give methyl 2,3,4,6-tetra-O-methyl-α- and β-glucopyranosides and a trimethoxy-monohydroxychalcone (XVI), $C_{18}H_{18}O_5$, mp 204—205°, UV λ_{max}^{EtOH} 326 nm, identical in all respects with a synthetic specimen of 2′,4,6′-trimethoxy-4′-hydroxychalcone,[12] mp 202—203°. Accordingly III should be the 4′-glucopyranoside of 2′,4,4′,6′-tetrahydroxychalcone or its methyl ether. Since III was acid hydrolyzed to give V,[13] the aglycone is regarded as 6′-(2′-) monomethyl ether.

Consequently, if the component glucose is assumed to be β-linked[14] D-enantiomer as is common in natural flavonoid glycosides, III is defined as 2′,4,4′-trihydroxy-6′-methoxychalcone 4′-β-D-glucopyranoside (dehydro-*para*-asebotin).

III was reported in 1944 by Zemplen, *et al.*[15] as an intermediate in the synthetic studies of phlorizin and related dihydrochalcone glucosides, but as far as we are aware, natural existence of III and V has never been reported.[16]

Experimental

Melting points were measured on Kofler block (Yazawa BY-1) and are uncorrected. Optical rotations, UV and mass spectra were respectively taken with JASCO automatic polarimeter DIP-SL, Hitachi spectrophotometer 181 and JMS-01-SG mass spectrometer at an ionizing voltage of 75 eV. 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. Abbreviations used are s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet. Unless otherwise indicated, thin-layer chromatographies (TLC) were carried out on "Kieselgel G nach Stahl" (Merck) and the spots were visualized by charing with 10% H_2SO_4. For column chromatographies "Kieselgel 60" (Merck) (a mixture (5: 1, w/w) of "0.063—0.200 mm" and "unter 0.08 mm") was employed. The solvent systems (v/v) were hexane–acetone (9: 1) (solv. 1), hexane–acetone (8: 2) (solv. 2), benzene–acetone (20: 1) (solv. 3), benzene–acetone (9: 1) (solv. 4), benzene–acetone (8: 2) (solv. 5), benzene–EtOAc (8: 2) (solv. 6) and $CHCl_3$–MeOH (8: 2) (solv. 7). The phenolic compounds and reducing sugars were examined on Avicel SF in the solvent systems of 40% (v/v) AcOH (visualizing reagent, 10% Na_2CO_3 (method 1) or diazotized *p*-sulfanilic acid (method 2)) and BuOH–pyridine–H_2O (3: 2: 1, v/v) (reagent, *p*-anisidine–HCl (method 3)). Gas–liquid chromatographies (GLC) were measured at column temperature 100° with Toyo GC-SD equipped with hydrogen flame detector and glass columns (4 mm × 1.5 m) packed with butanediol succinate (5%) on Chromosorb W (80—100 mesh). Carrier gas was nitrogen (50 ml/min).

Extraction and Isolation of Constituents——The fresh flowers of *G. affine* D. Don were percolated twice with MeOH. The MeOH extract was evaporated *in vacuo* to give a dark brown liquid, which was repeatedly extracted with benzene and then with EtOAc. The EtOAc extracts were dissolved in MeOH and treated with a MeOH solution of $Pb(OAc)_2$, giving precipitable (Fr. 1) and non-precipitable (Fr. 2) fractions. They were bubbled with H_2S, and the Pb salts were filtered off.

The MeOH solution of Fr. 1, which exhibited at least four spots at *Rf* 0.74 (brown), 0.58 (orange), 0.35 (yellow) and 0.26 (yellow) on TLC (method 1), was placed on a column of polyamide (C-200, Wako Pure Chemical Industries, Ltd.) and eluted with MeOH. The eluate was crystallized from MeOH, giving II, yellow needles, mp>230°, UV λ_{max}^{EtOH} nm(log ε): 255 (4.51), 352 (4.56). Its infrared (IR) spectrum was identical with that of authentic luteolin, and the acetate, colorless needles (from MeOH), showed mp 221—223°, either alone or on admixture with luteolin tetraacetate.

Fr. 2 revealed at least three spots (*Rf* 0.58 (orange), 0.35 (yellow) (tailing) and 0.22 (brown)) on TLC (method 1) and gave, by polyamide column chromatography, the compounds of *Rf* 0.58 and 0.35 respectively in TLC homogeneous state.

Crystallization of the compound of *Rf* 0.35 from H_2O furnished I as pale yellow needles (yield 0.3%), which melted at around 164—178°. Its IR spectrum was identical with that of I reported previously.[2]

12) N. Narasimhachari and T.R. Seshadri, *Proc. Indian Acad. Sci.*, **27A**, 223 (1948) [*C.A.*, **44**, 1493 (1950)].
13) *a*) T.R. Seshadri, "The Chemistry of Flavonoid Compounds," ed. by T.A. Geissman, Pergamon Press, Oxford, 1962, p. 156; *b*) M. Shimokoriyama, *ibid.*, p. 286.
14) In harmony with the finding of Mabry, *et al.*,[6] that the anomeric proton of 7-glucosidoxyflavanoids appears as complex multiplet, XV exhibited on its NMR spectrum the anomeric proton signal at 5.07 ppm (4.84 ppm at 100 MHz) as broad doublet. The spacing between the major peaks of them measured 9 Hz (*cf.* Experimental).
15) G. Zemplen, R. Bognar, and K. Thiele, *Chem. Ber.*, **77**, 446 (1944).
16) Birch, *et al.* (A.J. Birch and M. Salahuddin, *Tetrahedron Letters*, **1964**, 2211) reported isolation of naringenin and 5-O-methylnaringenin from *Xanthorrhoea preissii*, but the details have not appeared yet.

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The compound of *Rf* 0.58 was dissolved in hot 30% MeOH and then cooled. The yellow precipitates separated were removed by filtration and the filtrate was left stand for one week to give III as yellow needles. Yield 0.07%. It melted at 199—200° with the foregoing sintering at 140—160°, and showed $[\alpha]_D^{30}$ −85.2° (*c* 0.71, MeOH), UV λ_{max}^{EtOH} nm(log ε): 368 (4.51) (lit.,[15] changes to a glassy mass at 162° and melts at 199° (hydrated form) or 202—203° (nonhydrated form), $[\alpha]_D^{22}$ −38.6° (pyridine)). *Anal.* Calcd. for $C_{22}H_{24}O_{10}\cdot 1\frac{1}{2}H_2O$: C, 55.57; H, 5.72; H_2O, 5.7. Found: C, 55.57; H, 5.56; H_2O, 6.2. NMR ($(CD_3)_2SO$) δ ppm: 7.54 (2H, s, olefinic H), 7.56 and 6.82 (4H, two d, J=8 Hz, C_2–, C_6–, C_3– and C_5–H), 6.20 and 6.21 (2H, two d, J=2 Hz, $C_{3'}$– and $C_{5'}$–H), 3.89 (3H, s, OMe), 10.07 (H, s, C_4–OH), 13.07 (H, s, $C_{2'}$–OH). Dark brown with $FeCl_3$, negative to Mg–HCl and Zn–HCl tests.

III Hexaacetate (IV)——III (516 mg) was acetylated with Ac_2O (2 ml) and pyridine (2 ml) at room temperature overnight, and the product (*Rf* 0.18 (solv. 6)) was purified through column chromatography over silica gel (solv. 6) to give IV as colorless syrup (yield 74 mg), UV λ_{max}^{EtOH} nm(log ε): 306 (4.18). NMR ($CDCl_3$) δ ppm: 7.66 and 6.90 (2H, two d, J=17 Hz, olefinic H), 7.57 and 7.13 (4H, two d, J=8 Hz, C_2–, C_6–, C_3– and C_5–H), 6.54 and 6.44 (2H, two d, J=2 Hz, $C_{3'}$– and $C_{5'}$–H), 3.80 (3H, s, OMe), 2.30 and 2.17 (6H, two s, OAc×2 on aromatic ring), 2.08, 2.07 and 2.05 (12H, three s, OAc×4 on sugar moiety), 5.2, 4.2 and 3.8 (7H, m, protons on sugar moiety).

Acid Hydrolysis of III——III (284.3 mg) in H_2O (40 ml) and 1N HCl (10 ml) was heated on a water bath for 4 hr. After being cooled, the precipitates (V) (*Rf* 0.71 (yellow) (method 2)) were collected (yield 149.2 mg). Extraction of the filtrate with EtOAc gave further crop (18.5 mg) of V (*Rf* 0.71). Total yield 167.7 mg, 59%. In another hydrolysis III (57.7 mg) yielded V (38.0 mg, 66%).

The aqueous solution was neutralized by passing through a column of Amberlite IR 45 (OH^-), evaporated *in vacuo* to a syrup and examined by TLC (method 3). Only one spot was detected at *Rf* 0.24, identical with that of D-glucose. Quantitative determination of glucose after anthrone method,[3] 21.5 mg, 37%.

Aglycone (V)——V obtained above was recrystallized from dimethylsulfoxide–H_2O to give almost colorless needles, mp 256°, UV λ_{max}^{EtOH} nm(log ε): 228 (4.43), 284 (4.28) (lit.,[15] mp 263° with foregoing browning at 248°). *Anal.* Calcd. for $C_{16}H_{14}O_5$: C, 67.12; H, 4.93. Found: C, 67.05; H, 4.90. Red with Mg–HCl, reddish violet with Zn–HCl and no color with $FeCl_3$. NMR ($(CD_3)_2SO$) δ ppm: 5.36, 3.05 and 2.52 (3H, ABX system due to C_2– and C_3–H, J=4, 16 and 12 Hz), 7.33 and 6.81 (4H, two d, J=8 Hz, $C_{2'}$–, $C_{6'}$–, $C_{3'}$– and $C_{5'}$–H), 6.10 and 5.99 (2H, two d, J=2 Hz, C_8– and C_6–H), 3.76 (3H, s, OMe), 9.6 and 10.55 (2H, two broad s, OH×2).

V Diacetate (VI)——V (76 mg) was acetylated with Ac_2O (2 ml) and pyridine (2 ml) at room temperature overnight to afford VI as colorless needles (from $CHCl_3$–hexane), mp 174—175°, UV λ_{max}^{EtOH} nm(log ε): 285 (3.30) (lit.,[15] mp 176°). *Anal.* Calcd. for $C_{20}H_{18}O_7$: C, 64.86; H, 4.90. Found: C, 64.41; H, 4.81. NMR ($CDCl_3$) δ ppm: 5.31, 3.05 and 2.71 (3H, ABX system due to C_2– and C_3–H, J=5, 10 and 16 Hz), 7.24 and 6.91 (4H, two d, J=8 Hz, $C_{2'}$–, $C_{6'}$–, $C_{3'}$– and $C_{4'}$–H), 6.29 and 6.17 (2H, two d, J=2 Hz, C_8– and C_6–H), 3.81 (3H, s, OMe), 2.23 (6H, s, OAc×2).

V Dimethyl Ether (VII)——V (159 mg) in a mixture of dioxane (15 ml) and MeOH (5 ml) was methylated with ether solution of CH_2N_2 at room temperature overnight. The product (*Rf* 0.86 (violet), 0.82 (brown), 0.63 (red), 0.49 (yellow), 0.35 (dark violet) (solv. 5)) was chromatographed (solv. 3), and the fraction showing one spot at *Rf* 0.49 was crystallized from 70% MeOH to give VII as colorless needles (144 mg), mp 121°, UV λ_{max}^{EtOH} nm(log ε): 228 (4.24), 282 (4.02). *Anal.* Calcd. for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.58; H, 5.88. NMR ($CDCl_3$) δ ppm: 5.31, 3.06 and 2.67 (3H, ABX system due to C_2– and C_3–H, J=4, 12 and 17 Hz), 7.33 and 6.87 (4H, two d, J=8 Hz, $C_{2'}$–, $C_{6'}$–, $C_{3'}$– and $C_{5'}$–H), 6.03 and 6.09 (2H, two d, J=2 Hz, C_6– and C_8–H), 3.78 and 3.87 (9H, two s, OMe×3). Mixed melting point with authentic 4′,5,7-trimethoxyflavanone,[8] mp 121—122°, showed no depression and their IR spectra were superimposable.

V Diethyl Ether (VIII)——V (132 mg) in EtOH (100 ml) was treated with an ether solution of CH_3CHN_2[10] at room temperature for 4 hr. The reaction mixture (*Rf* 0.59 (major) and 0.47 (solv. 5)) was passed through a silica gel column (solv. 4), and the major product of *Rf* 0.59 was crystallized from benzene–hexane (1: 4, v/v) to provide VIII as pale yellow needles, mp 121—122°, UV λ_{max}^{EtOH} nm(log ε): 229 (4.58), 282 (4.38). *Anal.* Calcd. for $C_{20}H_{22}O_5$: C, 70.16; H, 6.48. Found: C, 70.24; H, 6.56. Mass Spectrum *m/e* (%): 342 (M^+) (25), 221 ($M^+-C_6H_4(OEt)$) (9), 194 ($C_6H_2(OEt)(OMe)(=O)(=C=O)^+$) (18), 149 (*m/e* 194−OEt) (100), 148 ($C_6H_4(OEt)(-CH=CH_2)^+$) (26).

Methylation of IX (Table I)——To a solution of IX (500 mg) in 20 ml of a solvent (anhyd. dioxane (purified after the procedure of Hess, *et al.*[17]), anhyd. MeOH and 70% MeOH), and ether solution (50 ml) of CH_2N_2 prepared from 5 g of nitrosomethylurea was added at 0°. The solution in 70% MeOH quickly consumed CH_2N_2 and, hence, further amount (50 ml) of the reagent was added. Each mixture was left stand for 3 and 24 hr at 0°, the excess of CH_2N_2 decomposed with AcOH, the solvent evaporated *in vacuo*, and the residue was chromatographed (solv. 4) over silica gel (20 g).

In anhyd. dioxane: Both products (reaction times 3 and 24 hr) exhibited six spots at *Rf* 0.85 (yellow) (XI), 0.82 (pink, trace), 0.73 (pink, trace), 0.57 (yellow) (X), 0.28 (black, trace) and 0.15 (black, trace) on

17) K. Hess and H. Frahm, *Chem. Ber.*, **71**, 2627 (1938).

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TLC (solv. 4), and gave X (70% (3 hr) and 47% (24 hr)) and XI (0.5% (3 hr) and 5% (24 hr)). X was crystallized from benzene as colorless needles, mp 148—149° (lit.,[9] mp 150°), UV λ_{max}^{EtOH} nm(log ε): 287 (4.28). Brown with $FeCl_3$. *Anal.* Calcd. for $C_{16}H_{14}O_5$: C, 67.12; H, 4.93. Found: C, 67.03; H, 4.85. NMR ($(CD_3)_2CO$) δ ppm: 5.49, 3.26 and 2.74 (3H, ABX system due to C_2– and C_3–H, J=4, 12 and 17 Hz), 7.41 and 6.90 (4H, two d, J=9 Hz, $C_{2'}$–, $C_{6'}$–, $C_{3'}$– and $C_{5'}$–H), 6.05 (2H, s, C_6– and C_8–H), 3.87 (3H, s, OMe), 8.52 (H, s, $C_{4'}$–OH), 12.13 (H, s, C_5–OH). XI was obtained as TLC homogeneous colorless needles, mp 106—110° (lit.,[9] mp 118°), identified with an authentic sample described later by cochromatography and mixed melting point determination.

In anhyd. MeOH: The intensities of the spot at *Rf* 0.85 (XI), 0.82, 0.73, 0.28 and 0.15 were enhanced and three additional spots were newly detected at *Rf* 0.65 (pink), 0.51 (black) and 0.38 (VII) (yellow).

From the reaction mixture for 3 hr, only XI (yield 42%) was isolated as colorless needles (from acetone–H_2O), mp 113—114° (lit.,[9] mp 118°), UV λ_{max}^{EtOH} nm(log ε): 226 (4.45), 288 (4.27). *Anal.* Calcd. for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37. Found: C, 68.08; H, 5.40. NMR ($CDCl_3$) δ ppm: 5.34, 3.15 and 2.72 (3H, ABX system due to C_2– and C_3–H, J=4, 12 and 17 Hz), 7.39 and 6.94 (4H, two d, J=9 Hz, $C_{2'}$–, $C_{6'}$–, $C_{3'}$– and $C_{5'}$–H), 6.06 (2H, s, C_6– and C_8–H), 3.82 and 3.84 (6H, two s, OMe×2), 12.03 (H, s, C_5–OH). The compounds of *Rf* 0.57 and 0.38 were respectively identified with X and VII by cochromatography on TLC.

The reaction mixture for 24 hr showed no spot of *Rf* 0.57 (X) on TLC, and XI (yield 33%), mp 113—114°, and VII (yield 31%), mp 119—121°, were provided.

In 70% MeOH: A significant enhancement of the intensities of the spots, *Rf* 0.82, 0.73, 0.65, 0.51, 0.28 and 0.15, was observed. The reaction for 3 hr provided XI (yield 22%) and VII (yield 48%). The reaction mixture for 24 hr revealed no spot of XI on TLC and gave only VII (yield 67%).

Ethylation of IX with Diazoethane to XII and XIII——To a solution of IX (500 mg) in anhyd. MeOH (20 ml) was added at 0° an ether solution of CH_3CHN_2 prepared from 5 g of N-ethyl-N-nitroso-N'-nitroguanidine.[10] CH_3CHN_2 was consumed completely in 3 hr at 0°. The reaction mixture (*Rf* 0.70 and 0.23 (solv. 2)) was chromatographed (solv. 4). The fraction of *Rf* 0.70 was crystallized from acetone–H_2O to give XII as colorless needles (yield 155 mg), mp 97—98°, UV λ_{max}^{EtOH} nm(log ε): 226 (4.47), 289 (4.29). *Anal.* Calcd. for $C_{19}H_{20}O_5$: C, 69.50; H, 6.14. Found: C, 69.58; H, 6.20. Brown with $FeCl_3$. NMR ($CDCl_3$) δ ppm: 5.34, 3.14 and 2.71 (3H, ABX system due to C_2– and C_3–H, J=5, 12 and 17 Hz), 7.36 and 6.92 (4H, two d, J=8 Hz, $C_{2'}$–, $C_{6'}$–, $C_{3'}$– and $C_{5'}$–H), 6.04 (2H, s, C_6– and C_8–H), 1.36 and 1.42 (3H×2, two t, J=6 Hz, $OCH_2C\underline{H}_3$×2), 4.03 and 4.05 (2H×2, two q, J=6 Hz, $OC\underline{H}_2CH_3$×2), 12.03 (H, s, C_5–OH). The fraction of *Rf* 0.23 was crystallized from benzene to afford XIII as colorless needles (yield 198 mg), mp 130—131°, UV λ_{max}^{EtOH} nm(log ε): 222 (4.45), 288 (4.30). *Anal.* Calcd. for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37. Found: C, 67.73; H, 5.30. Brown with $FeCl_3$. NMR ($CDCl_3$) δ ppm: 5.27, 3.07 and 2.66 (3H, ABX due to C_2– and C_3–H, J=5, 12 and 16 Hz), 7.19 and 6.75 (4H, two d, J=9 Hz, $C_{2'}$–, $C_{6'}$–, $C_{3'}$– and $C_{5'}$–H), 5.94 (2H, s, C_6– and C_8–H), 1.36 (3H, t, J=6 Hz, $OCH_2C\underline{H}_3$), 3.97 (2H, q, J=6 Hz, $OC\underline{H}_2CH_3$), 11.76 (H, s, C_5–OH). Methylation of XIII (0.31 g) in anhyd. MeOH (20 ml) with CH_2N_2 for 3 hr and subsequent crystallization of the product from MeOH provided XIV as colorless needles (yield 0.14 g), mp 111°, UV λ_{max}^{EtOH} nm(log ε): 224 (4.52), 289 (4.35). *Anal.* Calcd. for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.92; H, 5.73. NMR ($CDCl_3$) δ ppm: 5.29, 3.10 and 2.67 (3H, ABX due to C_2– and C_3–H, J=5, 12 and 18 Hz), 7.26 and 6.84 (4H, two d, J=8 Hz, $C_{2'}$–, $C_{6'}$–, $C_{3'}$– and $C_{5'}$–H), 5.95 (2H, s, C_6– and C_8–H), 1.38 (3H, t, J=6 Hz, $OCH_2C\underline{H}_3$), 3.96 (2H, q, J=6 Hz, $OC\underline{H}_2CH_3$), 3.78 (3H, s, OMe), 11.81 (H, s, C_5–OH). Mass Spectrum *m/e* (%): 314 (M^+) (74), 207 ($M^+-C_6H_4(OMe)$) (25), 180 ($C_6H_2(OEt)(OH)(=O)(=C=O)^+$) (19), 135 (*m/e* 180 −OEt) (19), 134 ($C_6H_4(OMe)(-CH=CH_2)^+$) (100).

Methylation of XII to VIII——Because of the poor solubility in 70% MeOH, XII (288 mg) was dissolved in a mixture of ether (10 ml) and 70% MeOH (10 ml), and methylated with an ether solution of CH_2N_2 prepared from 5 g of nitrosomethylurea at 0° for 24 hr. The product (*Rf* 0.70 (pink), 0.65 (pink), 0.53 (brown), 0.48 (yellow), 0.40 (pink), solv. 4) was chromatographed (solv. 4). The fraction of *Rf* 0.48 (252 mg) was crystallized from 70% MeOH to give VIII as colorless needles, mp 121—122°. No coloration with $FeCl_3$. *Anal.* Calcd. for $C_{20}H_{22}O_5$: C, 70.16; H, 6.48. Found: C, 70.16; H, 6.46. On admixture with VIII derived from V, it did not depress the melting point and their IR spectra were identical.

III Hexamethyl Ether (XV)——A mixture of III (978 mg), Me_2SO_4 (3.38 g) and K_2CO_3 (12.0 g) in anhydrous acetone (50 ml) was refluxed for 24 hr. The reaction mixture was filtered and evaporated to dryness. The residue (1.63 g) was stirred in dimethylformamide (20 ml) with MeI (2 g) and Ag_2O (3.2 g) for 24 hr. After removal of Ag salt, the reaction mixture was evaporated *in vacuo* and the residue (*Rf* 0.63, 0.45, 0.39, 0.32 (major), 0.23—0.09 (solv. 4)) was chromatographed (solv. 4). Crystallization of the fraction of *Rf* 0.32 from 70% MeOH gave XV (yield 329 mg) as pale yellow needles, mp 112—113°, $[\alpha]_D^{24}$ −18.6° (*c* 0.98, $CHCl_3$), UV λ_{max}^{EtOH} nm(log ε): 327 (4.41). *Anal.* Calcd. for $C_{28}H_{36}O_{10}$: C, 63.14; H, 6.81. Found: C, 63.27; H, 6.80. NMR ($CDCl_3$) δ ppm: 7.42 and 6.88 (2H, two d, J=17 Hz, olefinic H), 7.59 and 6.98 (4H, two d, J=9 Hz, C_2–, C_6–, C_3– and C_5–H), 6.47 (2H, s, $C_{3'}$– and $C_{5'}$–H), 3.46, 3.63, 3.72, 3.77, 3.83 and 3.92 (3H×6, six s, OMe×6), 5.07 (H, broad d, distance between the major peaks, 9 Hz, anomeric H). On a spectrum taken at 100 MHz (at 23° and 50°), the anomeric proton was detected as broad doublet (J=about 9 Hz) centering at 4.84 ppm.

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Methanolysis of XV——A solution of XV (102 mg) in anhyd. MeOH containing 1% HCl was refluxed for 2 hr. The reaction mixture was made alkaline with 10% NaOH, concentrated to half volume, diluted with H_2O, and extracted with $CHCl_3$. The organic layer gave a violet syrup (46 mg, neutral fraction). The aqueous layer was acidified with 10% HCl and extracted with $CHCl_3$ to give acidic fraction as an orange syrup (42 mg). The neutral fraction showed on TLC (solv. 2) two spots at *Rf* 0.23 (black) and 0.13 (black, major), and on GLC two peaks at t_R 1.7 and 2.4 (major), identical respectively with the *Rf* and t_R values of methyl 2,3,4,6-tetra-O-methyl-β- and α-D-glucopyranosides.

The acidic fraction consisted of a compound (*Rf* 0.26 (solv. 4)) accompanied by a trace amount of methyl 2,3,4,6-tetra-O-methyl-α-glucopyranoside (*Rf* 0.36). Column chromatographic purification gave the homogeneous compound of *Rf* 0.26, which was crystallized from acetone–hexane to yield XVI as yellow plates, mp 204—205°, UV λ_{max}^{EtOH} nm(log ε): 326 (4.31). *Anal.* Calcd. for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.41; H, 5.71. NMR ($(CD_3)_2SO$) δ ppm: 7.20 and 6.76 (2H, two d, J=18 Hz, olefinic H), 7.60 and 6.96 (4H, two d, J=8 Hz, C_2–, C_6–, C_3– and C_5–H), 6.13 (2H, s, $C_{3'}$– and $C_{5'}$–H), 3.67 and 3.81 (9H, two s, OMe×3). On admixture with an authentic specimen, mp 202—203°, prepared by methylation of naringin with Me_2SO_4 and NaOH followed by acid hydrolysis of the product,[12] it did not depress the melting point. IR, UV and NMR spectra were identical with those of the authentic specimen.

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