*Phytochemistry*, 1977, Vol. 16, pp. 1265–1267. Pergamon Press. Printed in England.

# BITTER PHENYLPROPANOID GLYCOSIDES FROM *CONANDRON RAMOIDIOIDES*

GENICHIRO NONAKA and ITSUO NISHIOKA

Faculty of Pharmaceutical Sciences, Kyushu University, Maidashi, Fukuoka, Japan

(*Received* 16 *December* 1976)

**Key Word Index**—*Conandron ramoidioides*; Gesneriaceae; bitter glycosides; conandroside; acteoside; acyl migration; caffeic acid; sugar esters.

**Abstract**—A new bitter glycoside, conandroside and a known glycoside, acteoside were isolated from *Conandron ramoidioides*. On the basis of the chemical and spectral evidence, conandroside was shown to be 2-(3′,4′-dihydroxyphenyl)-ethanol 1-*O*-β-D-xylosyl-(1 → 3)-β-D-(4-caffeyl)-glucoside.

The MeOH extract of the dried whole plant was partitioned between EtOAc and $H_2O$, and the $H_2O$ layer was then extracted with *n*-BuOH, which, containing the bitter principles, displayed two spots positive to $FeCl_3$ reagent on TLC. Repeated polyamide and silica gel column chromatography of the *n*-BuOH extract afforded an inseparable mixture of conandroside (**1**) and acteoside (**2**) previously isolated from *Syringa vulgaris* (Oleaceae) [1]. Purification through the methyl ethers followed by repeated silica gel column chromatography, afforded individual pure tetramethyl ethers.

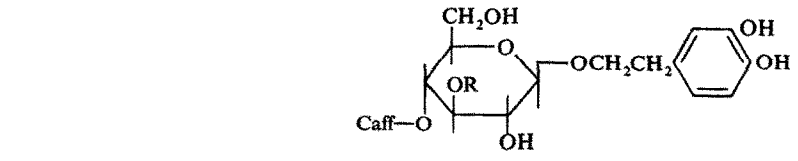
Conandroside tetra-*O*-methyl ether (**3**) thus obtained was, from spectral properties, a glycoside having a conjugated ester group. It afforded xylose, glucose, 3,4-dimethoxyphenethyl alcohol, and caffeic acid on complete hydrolysis with alkali and then with acid. Methylation of **3** by the Hakomori procedure [2] provided a colourless amorphous octa-*O*-methyl ether (**4**) and dimethyl caffeic acid methyl ester. **4** shows no absorption bands due to the hydroxyl and conjugated ester groups on the IR and UV spectra; the MS exhibits the molecular ion peak at *m/e* 560 and peak due to the terminal permethylated xylose residue at *m/e* 175. Methanolysis of **4** with 6% HCl–MeOH furnished methyl 2,3,4-tri-*O*-methyl-xylopyranoside and methyl 2,4,6-tri-*O*-methyl-glucopyranoside, which were identified with authentic samples by TLC and GLC. These results indicate that the xylose residue is attached to the C-3 hydroxyl group of glucose moiety.

The location of dimethyl caffeic acid was deduced as follows. The NMR spectrum of **3** exhibits the well-resolved triplet signal at δ5.05 (1H, *t*, *J* = 8.5 Hz) assignable to the ester-bearing methine proton. However, it is not possible to make assignments for protons on neighbouring carbon atoms because they lie within a complicated region. Mild acid hydrolysis of **3** with 2N $H_2SO_4$ afforded a partially hydrolyzed product (**5**), which shows in the IR spectrum absorption due to the conjugated ester group at 1705 and 1630 $cm^{-1}$. The MS of **5** exhibits the molecular ion peak at *m/e* 534, in addition to the prominent peaks due to the dimethyl caffeic acid at *m/e* 208 and 191. These facts indicate that only the terminal xylose is liberated from **3** by the mild acid hydrolysis, and that dimethyl caffeic acid is attached to the glucose moiety. The NMR spectrum of **5** shows a signal at δ 4.99 (1H, *t*, *J* = 8.5 Hz) due to the proton of the methine group bearing the ester group. The assignment of this triplet was performed by double resonance experiments. Irradiation of the signal at δ4.99 altered the multiplet signals near δ3.48 and 3.76, but did not change the doublet due to the anomeric proton at δ4.34. Irradiation of the signal near δ3.48 changed the anomeric proton signal at δ4.34 to a singlet and the triplet signal at δ4.99 to a broad doublet. Since the acyl group is absent from the C-3 hydroxyl group, there are two one-proton signals overlapping near δ3.48 which are assignable to the C-2 and C-3 (or C-5) protons. Thus, dimethyl caffeic acid must be linked to the C-4 hydroxyl group in glucose moiety.

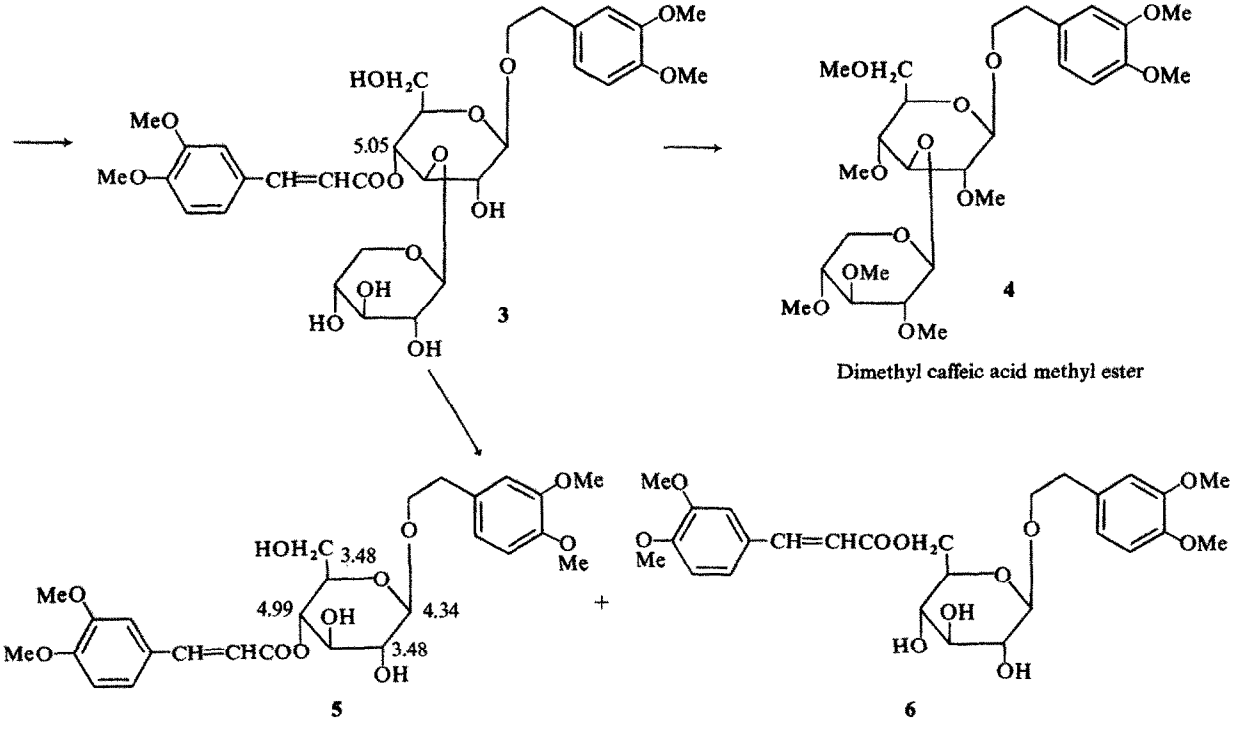
The above-mentioned mild acid hydrolysis afforded as another partial hydrolysate (**6**), which displayed very similar IR and MS spectra to those of **5**, whereas in the NMR spectrum it exhibits the signal assignable to the protons of the methylene group bearing the ester group at δ4.44 (2H, *d*, *J* = 3 Hz). Since the two-proton signal can be assigned to the C-6 methylene protons of glucose, dimethyl caffeic acid is combined with the C-6 hydroxyl group, and this shows the acyl migration took place during acid hydrolysis. Acyl migration of caffeic acid from the C-4 hydroxyl group to the C-6 hydroxyl group in the glucose moiety was also reported in acteoside during partial hydrolysis and permethylation with Kuhn's method [1].

The configurations of the sugar linkage were deduced to be β-forms for xylose and glucose by the NMR spectrum of **3** which exhibits the signals due to the anomeric protons of xylose and glucose as doublets at δ4.52 (*J* = 7 Hz) and δ4.37 (*J* = 7.5 Hz), respectively. Since no signal due to the methoxyl group is observed in the NMR spectrum of crude conandroside, conandroside must be 2-(3′,4′-dihydroxyphenyl)-ethanol 1-*O*-β-D-xylopyranosyl-(1 → 3)-β-D-(4-caffeyl)-glucopyranoside.

The tetramethyl ether **7** of **2** was similarly studied (see Experimental) and the structure of **2** was determined as 2-(3′,4′-dihydroxyphenyl)-ethanol 1-*O*-α-L-rhamnopyranosyl-(1 → 3)-β-D-(4-caffeyl)-glucopyranoside, which is that of acteoside.



1 R = Xyl
2 R = Rha



Dimethyl caffeic acid methyl ester

## EXPERIMENTAL

Mps are uncorr. NMR spectra were measured at 100 MHz and chemical shifts are given on $\delta$ (ppm) scale with TMS as the internal standard. GLC was run using glass column (2.0 × 4 mm$\phi$) packed with 5% 1,4-butanediol succinate on Shimalite W (60–80 mesh). PC for sugars was carried out using aniline hydrogen phthalate for staining. TLC was conducted on Kieselgel G nach Stahl using $FeCl_3$ reagent and 10% $H_2SO_4$ as the detector. Column chromatography was carried out with Kieselgel (70–200 mesh), polyamide C-200 and Sephadex LH-20 (25–100μ).

*Isolation of bitter principles.* The dried and finely powdered herbs (14.5 kg) of *Conandron ramoidioides* were extracted with MeOH ( × 4) at room temp. The MeOH extracts were combined and the solvent was evaporated to give a dark brown residue, which was partitioned between EtOAc and $H_2O$. The aq. layer was then extracted with *n*-BuOH to give a brown *n*-BuOH extract (444 g). The *n*-BuOH extract (230 g) in $H_2O$ was passed through a polyamide (600 g) column and successive elution with $H_2O$ to afford a mixture of **1** and **2** (18 g), which was further purified by repeated $SiO_2$ column chromatography (EtOAc–MeOH), and with MeOH to give a flavone. A mixture of **1** and **2** in dry $Me_2CO$ was refluxed with $K_2CO_3$ and $Me_2SO_4$ for 1 hr. After cooling, excess $K_2CO_3$ was filtered off, and the filtrate was condensed to remove $Me_2CO$. The oily residue was chromatographed over $SiO_2$, and elution with $CHCl_3$–MeOH (47:3) yielded a colourless powder (**3**), mp 137–139°, $[\alpha]_D^{23}$ − 50.0° (*c* 1.0, MeOH). (Found: C, 56.32; H, 6.37. C, H, O, H O requires: C, 56.13; H, 6.48%). $\lambda_{max}^{MeOH}$ nm($\varepsilon$): [illegible] (16700). $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3440, 1705, 1632, 1600. NMR ($CDCl_3$) ppm: 2.88 (*t*, $J$ = 7.5 Hz, Ar—$CH_2$—), 3.80, 3.82 (*s*, OMe), 3.86 (*s*, 2 × OMe), 4.37 (*d*, $J$ = 7.5 Hz, anomeric H of glucoside), 4.52 (*d*, $J$ = 7 Hz), anomeric H of xyloside), 5.05 (*t*, $J$ = 8.5 Hz, —COOCH<), 6.26 (*d*, $J$ = 16 Hz, Ar—CH=CH—), 6.73–7.10 (6H, *m*, aromatic H), 7.61 (*d*, $J$ = 16 Hz, Ar—CH=CH—). Further elution with $CHCl_3$–MeOH (47:3) gave the tetra-*O*-methyl ether (**7**) of **2** as a colourless amorphous powder, $[\alpha]_D^{30}$ − 77.0° (*c* 1.0, MeOH), (Found: C, 56.95; H, 6.87. $C_{33}H_{44}O_{15}\cdot H_2O$ requires: C, 56.72; H, 6.64%). $\lambda_{max}^{MeOH}$ nm($\varepsilon$): 285 (13800), 322 (16800). $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3420, 1712, 1632, 1600. NMR ($CDCl_3$). ppm: 1.16 (*d*, $J$ = 7 Hz, rhamnose $C_6$-Me), 2.85 (*t*, $J$ = 7 Hz, Ar—$CH_2$—), 3.80, 3.81 (*s*, OMe), 3.96 (*s*, 2 × OMe), 4.34 (*d*, $J$ = 7 Hz, anomeric H of glucoside), 5.10 (*t*, $J$ = 8.5 Hz, —COOCH<), 5.24 (broad *s*, anomeric H of rhamnoside), 6.32 (*d*, $J$ = 16 Hz, Ar—CH=CH—), 6.72–7.12 (6H, *m*, aromatic H), 7.68 (*d*, $J$ = 16 Hz, Ar—CH=CH—).

*Alkaline treatment of* **3** *followed by acid hydrolysis.* **3** (50 mg) in aq. 2% NaOH was kept at room temp. for 50 min. The reaction mixture was acidified with 4N HCl and the resulting ppt. was extracted with $Et_2O$. The $Et_2O$ soln was washed, dried and evaporated to give colourless needles, mp 181–182° (8 mg), identified as dimethyl caffeic acid (mmp). The aq. layer was extracted with *n*-BuOH. The *n*-BuOH layer was washed and evaporated to give colourless granules (16 mg). A soln of this alkaline hydrolysate (9 mg) in aq. 4N HCl (1 ml) was heated on a water bath for 1 hr. After cooling, the mixture was extracted with $Et_2O$. The $Et_2O$ layer was washed, dried and evaporated to give a light yellow oil, identified as 3,4-dimethoxyphenethyl alcohol (TLC, IR), while the aq. layer was neutralized with

$Ag_2CO_3$ and ppt. was filtered off. The filtrate was evaporated and the residue was examined by PC [*n*-BuOH–HOAc–$H_2O$ (4:1:5, upper layer)] to identify xylose ($R_f$: 0.29) and glucose ($R_f$: 0.21).

*Methylation of* **3** *by the Hakomori method.* **3** (40 mg) in DMSO (2 ml) was treated with dimsyl carbanion soln (4 ml, prepared from NaH (120 mg) and DMSO (5 ml) and the soln was kept stirred at room temp. for 30 min. To this soln was added MeI (1 ml) and stirred for further 30 min. The reaction mixture was poured onto ice and extracted with $CHCl_3$. The $CHCl_3$ soln was washed, dried and evaporated to afford a colourless syrup, which was chromatographed over $SiO_2$. Elution with benzene–$Me_2CO$ (9:1) yielded dimethylcaffeic acid methyl ester, mp 67°, and elution with benzene–$Me_2CO$ (4:1) afforded a colourless amorphous octa-*O*-methyl ether (27 mg), **4**, $[\alpha]_D^{23}$ − 40.0° (*c* 0.5, MeOH). MS *m/e*: 560.2833 ($M^+$, Calcd. for $C_{27}H_{44}O_{12}$: 560.2855), 175 ($C_8H_{15}O_4^+$, terminal permethylated xylose residue). $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1605, 1593. NMR ($CDCl_3$) ppm: 2.89 (*t*, *J* = 7 Hz, Ar—$CH_2$—), 3.37, 3.51, 3.56, 3.59, 3.81, 3.83 (*s*, OMe), 3.44 (*s*, 2 × OMe), 4.24, (*d*, *J* = 7.5 Hz, anomeric H of glucoside), 4.70 (*d*, *J* = 7 Hz, anomeric H of xyloside), 6.73 (3H, *s*, aromatic H).

*Methanolysis of* **4**. **4** (10 mg) in 6% HCl–MeOH (1 ml) was refluxed for 3 hr, and the mixture was neutralized with $Ag_2CO_3$. The ppt. was filtered off and the filtrate was evaporated. The residue was subjected to TLC [EtOAc–MeOH (50:1)] and to GLC (column temp.: 185°, $N_2$ flow rate: 90 ml $min^{-1}$) to identify methyl 2,3,4-tri-*O*-methylxylopyranoside [$R_f$: 0.87, $t_R$ (min): 1.0 (minor), 1.2 (major)] and methyl 2,4,6-tri-*O*-methyl-glucopyranoside [$R_f$: 0.32, $t_R$ (min): 5.1 (minor), 7.2 (major)].

*Partial hydrolysis of* **3**. **3** (60 mg) in N $H_2SO_4$ (3 ml) ($H_2O$–EtOH = 1:1) was refluxed at 90° for 3 hr, the EtOH removed and diluted with $H_2O$. The mixture was extracted with EtOAc (×3). The EtOAc layer was washed, dried and evaporated to yield a colourless syrup, which was chromatographed over Si gel (10 g) eluting with $CHCl_3$–MeOH mixture. The elution with $CHCl_3$–MeOH (97:3) gave a colourless amorphous powder (**5**, 21 mg), $[\alpha]_D^{19}$ + 12.7° (*c* 0.36, MeOH). MS *m/e*: 534.2107 ($M^+$, Calcd. for $C_{27}H_{34}O_{11}$: 534.2102), 344.1453 ($C_{16}H_{24}O_8^+$: 344.1471), 210.0896 ($C_{11}H_{14}O_4^+$: 210.0892), 208.0730 ($C_{11}H_{12}O_4^+$: 208.0736), 195.0979 ($C_{11}H_{15}O_3^+$: 195.1020), 191.0735 ($C_{11}H_{11}O_3^+$: 191.0708), 182.0992 ($C_{10}H_{14}O_3^+$: 182.0943), 164.0835 (base peak, $C_{10}H_{12}O_2^+$: 164.0837). $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3590, 3500, 1705, 1630, 1598. NMR ($CDCl_3$) ppm: 2.91 (*t*, *J* = 7 Hz, Ar—$CH_2$—), 3.84, 3.86 (*s*, $OCH_3$), 3.90 (*s*, 2 × OMe), 4.34 (*d*, *J* = 7.5 Hz, anomeric H of glucoside), 4.99 (*t*, *J* = 8.5 Hz, —COOCH<), 6.29 (*d*, *J* = 16 Hz, Ar—C<u>H</u>=CH—), 6.76–712 (6H, *m*, aromatic H), 7.66 (*d*, *J* = 16 Hz, Ar—CH=C<u>H</u>—). Further elution with $CHCl_3$–MeOH (19:1) gave a colourless amorphous powder (**6**, 10 mg). MS *m/e*: 534 ($M^+$), 344, 210, 208, 195, 191, 182, 164 (base peak). $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3600, 3470, 1705, 1630, 1602. NMR ($CDCl_3$) ppm: 2.89 (*t*, *J* = 7 Hz, Ar—$CH_2$—), 3.79, 3.81, 3.87, 3.88 (*s*, OMe, 4.44 (*d*, *J* = 3 Hz, —$COOCH_2$—), 6.34 (*d*, *J* = 16 Hz, Ar—C<u>H</u>=CH—), 6.72–7.20 (6H, *m*, aromatic H), 7.65 (*d*, *J* = 16 Hz, Ar—CH=C<u>H</u>—).

*Alkaline hydrolysis of* **7**. **7** (96 mg) in 0.5N NaOMe–MeOH (3 ml) was refluxed for 15 min. The mixture was diluted with $H_2O$ and acidified with 35% HCl. The acidic soln was extracted with $Et_2O$ and then *n*-BuOH. The $Et_2O$ soln gave colourless needles, mp 182–183°, $\lambda_{max}^{MeOH}$ nm(ε): 286 (20400), 311 (21600), $\nu_{max}^{KBr}$ $cm^{-1}$: 1680, 1625, 1580, identified as dimethyl caffeic acid with an authentic sample (TLC, UV, IR, mmp). The *n*-BuOH soln gave colourless granules (12 mg), mp 184–186°. (Found: C, 51.50; H, 6.81. $C_{22}H_{34}O_{12}.H_2O$ requires: C, 51.96; H, 7.14. $\lambda_{max}^{MeOH}$ nm(ε): 275 (7000). $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3400, 1594. NMR ($Me_2CO$-$d_6$) ppm: 1.27 (*d*, *J* = 7 Hz, rhamnose $C_6$-Me), 2.83 (*d*, *J* = 7 Hz, Ar—$CH_2$—), 3.75, 3.77 (*s*, OMe), 5.16 (broad *s*, anomeric H of rhamnoside), 6.78–6.88 (3H, *m*, aromatic H).

*Alkaline treatment of* **7** *followed by acid hydrolysis.* Using the same procedure as with **3** yielded dimethyl caffeic acid, 3,4-dimethoxy phenethyl alcohol, rhamnose and glucose. Methylation of **7** by Hakomori's method gave dimethyl caffeic acid methyl ester, and a colourless amorphous octa-*O*-methyl ether, (**8**) $[\alpha]_D^{26}$ − 41.8° (c 1.59, MeOH). MS *m/e*: 574.2952 ($M^+$, Calcd for $C_{28}H_{46}O_{12}$: 574.2989), 189 ($C_9H_{17}O_4^+$, terminal permethylated rhamnose residue). $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1605, 1595. NMR ($CDCl_3$) ppm: 1.18 (*d*, *J* = 7 Hz, rhamnose $C_6$-Me), 3.40, 3.45, 3.52, 3.56 (*s*, OMe), 4.25 (*d*, *J* = 7.5 Hz, anomeric H of glucoside), 5.31 (broad *s*, anomeric H of rhamnoside), 6.76 (*s*, aromatic H). Methanolysis of **8** yielded methyl 2,3,4-tri-*O*-methylrhamnopyranoside [$R_f$: 0.72, $R_t$ (min): 1:1] and methyl 2,4,6-tri-*O*-methyl-glucopyranoside [$R_f$: 0.32, $t_R$ (min): 6.7 (minor), 9.5 (major)].

*Partial hydrolysis of* **7**. **7** (170 mg) in 2N $H_2SO_4$ (10 ml) ($H_2O$–EtOH 1:1) was heated at 90° for 2.5 hr, EtOH removed and the mixture was diluted with $H_2O$ and extracted with EtOAc (×3). The colourless residue (100 mg) from this extract was chromatographed over Si gel with $CHCl_3$–MeOH (19:1) to afford the partial hydrolysate as a colourless amorphous powder (55 mg), whose IR ($CHCl_3$) and NMR spectra were identical with **5**. The elution with $CHCl_3$–MeOH (19:1) gave a colourless amorphous powder (23 mg), which was identified as **6**.

*Methylation of* **7** *by the Kuhn method.* Methylation afforded a colourless amorphous powder (**9**), $[\alpha]_D^{28}$ − 45.6° (*c* 0.68, MeOH). MS *m/e*: 750 ($M^+$), 189 ($C_9H_{17}O_4^+$, terminal permethylated rhamnose residue), 164 (base peak). $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1710, 1632, 1602. NMR ($CDCl_3$) ppm: 1.30 (*d*, *J* = 7 Hz, rhamnose $C_6$-Me, 2.94 (*t*, *J* = 7 Hz, Ar—$CH_2$—), 3.50–3.56 (*s*, 5 × Me), 3.84, 3.85 (*s*, OMe), 3.92 (*s*, 2. × OMe), 4.33 (*d*, *J* = 7.5 Hz, anomeric H of glucoside), 4.42 (*d*, *J* = 3 Hz, —$COOCH_2$—), 5.34 (broad *s*, anomeric H of rhamnoside), 6.36 (*d*, *H* = 16 Hz, Ar—C<u>H</u>=CH—), 6.76–7.12 (6H, *m*, aromatic H), 7.68 (*d*, *J* = 16 Hz, Ar—CH=C<u>H</u>—). Alkaline treatment of **9** followed by methanolysis gave methyl 2,3,4-tri-*O*-methylrhamnopyranoside [$R_f$: 0.72, $t_R$ (min): 1.1] and methyl 2,4-di-*O*-methylgluco-pyranoside [$t_R$ (min): 16.9 (minor), 24.1 (major)]. Methylation of **5** by the Kuhn method gave a colourless amorphous powder (**10**, 21 mg), $[\alpha]_D^{28}$ − 10.0° (*c*, 0.25, MeOH). MS *m/e*: 564 ($M^+$), 164 (base peak). $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1710, 1632, 1602. NMR ($CDCl_3$) ppm: 2.91 (*t*, *J* = 7 Hz, Ar—$CH_2$—), 3.51, 3.54, 3.64, 3.83, 3.84 (*s*, OMe), 3.91 (*s*, 2 × OMe), 4.30 (*d*, *J* = 7.5 Hz, anomeric H of glucoside), 4.40 (*d*, *J* = 3 Hz, —$COOCH_2$—), 6.34 (*d*, *J* = 16 Hz, Ar—C<u>H</u>=CH—), 6.75–7.10 (6H, *m*, aromatic H), 7.64 (*d*, *J* = 16 Hz, Ar—CH=C<u>H</u>—). Alkaline treatment of **10** followed by methanolysis provided methyl 2,3,4-tri-*O*-methyl-glucopyranoside [$R_f$: 0.36, $t_R$ (min): 5.3 (minor), 7.3 (major)].

*Acknowledgements*—The authors are deeply grateful to Prof. T. Kawasaki of this University and Dr. H. Okabe of Fukuoka University for generous gifts of authentic sugar samples. Thanks are also due to Prof. O. Tanaka of Hiroshima University for CMR spectral measurement and to Miss Y. Miyoshi for technical assistance. The authors are indebted to Mr. H. Matsui, Mr. Y. Tanaka, Miss M. Kawamura and Miss K. Soeda for IR, UV, NMR and MS measurements, and to the members of the Central Analysis Room of this University for micro-analyses.

## REFERENCES

1. Birkofer, L., Kaiser, C. and Thomas, U. (1968) *Z. Naturforsch.* 1051.
2. Hakomori, S. (1964) *J. Biochem.* **55**, 205.
3. Kuhn, R., Trischmann, H. and Low, I. (1955) *Angew. Chem.* **67**, 32.