# Isolation of 5,7-Dihydroxy-2-methylchromone and its 7-*O*-Glycosides from *Adina rubescens*

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5.7-Dihydroxy-2-methylchromone (1a) has been isolated from *Adina rubescens* together with its 7-*O*-β-D-glucoside (1b) and two 7-*O*-pentosylglucosides (1c).

In comparison with the ubiquitous flavones and isoflavones, there is a paucity of other naturally occurring chromones; these include a few 2-hydroxymethyl derivatives and about twenty 2-methylchromones, ten of which have come from only two plants, *Amni visnagi* (Umbelliferae) and *Eugenia caryophyllata* (Myrtaccae).[1,2] We now report the isolation of four new natural products from *Adina rubescens* Hemsl. (Rubiaceae) which have been identified as 5,7-dihydroxy-2-methylchromone (*O*-demethyleugenin) (1a) and its 7-*O*-glycosides. One of the latter has been shown to be the β-D-glucoside (1b), the other two being disaccharides, partially characterised as (1c).

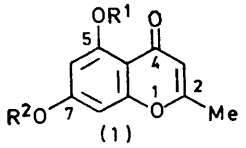
During the chromatographic fractionation of *A. rubescens* bark extract a small amount was obtained of an unknown material, $C_{10}H_8O_4$, m.p. 275—280° with characteristic u.v. maxima at 228, 248, 256, 293, and 310 nm, shifting to 264 and 330 nm on addition of alkali. Broad i.r. absorption between 3 200 and 2 400 $cm^{-1}$ suggested strong hydrogen bonding. The position of a strong carbonyl band at 1 660 $cm^{-1}$ tended to exclude a coumarin structure (2), also incompatible with ions such as *m/e* 152 in the mass spectrum (see Scheme). A number of metastable ions greatly assisted the interpretation of the fragmentation pattern, which suggested a general chromone structure although precise positions of hydroxy- and methyl substituents remained unknown.[3] From the very simple n.m.r. spectrum the number of possibilities could be reduced to two [(1a) and (3)] which were distinguished by the marked bathochromic shift of the u.v. maxima to 257, 303, and 357 nm on addition of aluminium chloride. Since this change was unaffected by addition

[1] F. M. Dean, 'Naturally Occurring Oxygen Ring Compounds,' Butterworths, London, 1963.

[2] H. Schmid in 'Progress in the Chemistry of Organic Natural Products,' vol. XI, Springer-Verlag, Berlin, 1954.

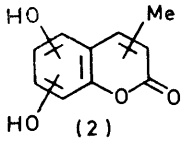
[3] M. M. Bodawi, M. B. E. Fayez, F. A. Bryce, and R. P. Reed, *Chem. and Ind.*, 1966, 498.

of acid, the complex formed must be particularly stable, involving the C-5 hydroxy-group with the C-4 carbonyl

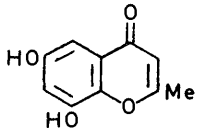


(1)

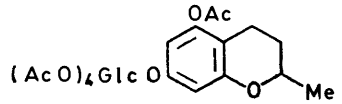
| | $R^1$ | $R^2$ |
|---|---|---|
| a; | H | H |
| b; | H | β-D-Glc |
| c; | H | β-D-Glc(OH)$_3$·OPent(OH)$_3$ |
| d; | Me | β-D-Glc |
| e; | Me | H |
| f; | Me | Ac |
| g; | Ac | β-D-Glc(OAc)$_4$ |
| h; | H | β-D-Glc(OAc)$_4$ |
| i; | Ac | β-D-Glc(OAc)$_3$·O Pent(OAc)$_3$ |



(2)

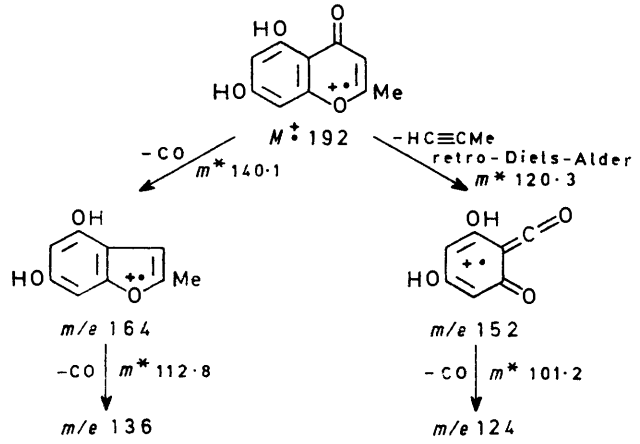


(3)



(4)

group as chelating ligand. Final confirmation that the natural product was 5,7-dihydroxy-2-methylchromone




SCHEME

(1a) came from comparison of physical data with those reported for synthetic material.[4]

Extraction of the fresh leaves of *A. rubescens* afforded a quantity of another chromone, m.p. 245—248° for which the molecular formula $C_{16}H_{18}O_9$ was established by mass measurement. The u.v. spectrum was essentially the same as that of the dihydroxychromone (1a), n.m.r. signals corresponding to H-3, -6, and -8, and 2-Me were apparent, and apart from the molecular ion at *m/e* 354, the mass spectrum was also very similar with distinctive fragments at *m/e* 192, 164, 152, and 124. From the difference in formulae the new compound was almost certainly a chromone hexoside.

Acetylation under vigorous conditions furnished a penta-acetate m.p. 124—129°, whose mass spectrum showed fragments characteristic of a hexoside tetra-acetate at *m/e* 331, 169, 127, and 109. Four acetate signals in the n.m.r. spectrum between τ 7.9 and 8.1 and other resonances at τ 4.6—5.2 (1′-, 2′-, 3′-, and 4′-H), 5.82 (6′-$H_2$), and 6.10 (5′-H), assigned with the aid of decoupling, were only consistent with a glucopyranoside unit.[5] Acetylation under mild conditions afforded an amorphous tetra-acetate, $[\alpha]_D^{25}$ −37°, which differed in lacking a phenolic acetate signal at τ 7.76, and showing a hydroxy-proton signal at τ 2.67. Removal of the sugar from the parent compound with β-glucosidase established that it was a β-D-glucoside of 5,7-dihydroxy-2-methylchromone.

It remained to find the position of the glucosidic linkage. A bathochromic shift in the u.v. spectrum on addition of aluminium chloride to a solution of the glycoside was similar to that observed previously for the aglycone, and strongly suggested that the 5-hydroxy-group was free. Prolonged treatment of the glycoside with diazomethane gave a monomethyl derivative (1d), from which the sugar was cleaved with acid to yield the methylated aglycone (1e), and acetylation gave the acetoxymethoxychromone (1f). Since none of the u.v. spectra was affected by aluminium chloride in the above manner, all these compounds must be 5-methoxy-derivatives, and this confirmed that the sugar must be attached to the 7-hydroxy-group. The glycoside is thus 7-β-D-glucosyloxy-5-hydroxy-2-methylchromone (1b).

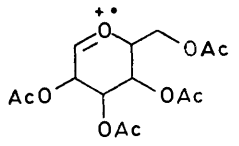
Gel-permeation chromatography of the highly polar components of the *A. rubescens* bark extract afforded a fraction showing the dihydroxychromone u.v. chromophore. Acetylation and separation by t.l.c. gave two isomeric glycoside acetates with very similar properties. Both had u.v. and i.r. spectra similar to those of the 7-glucosyloxychromone penta-acetate (1g), and n.m.r. spectra showed the substitution pattern around the chromone ring to be the same. However, signals for a phenolic acetate at τ 7.6, six acetates between τ 7.8 and 8.0, and other appropriate signals suggested a disaccharide unit. The u.v. behaviour of the parent glycosides in the presence of aluminium chloride again indicated that the 5-hydroxy-group was free, and the sugar was thus linked to the 7-position.

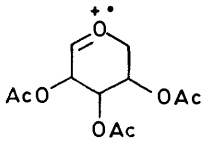
Support for a disaccharide unit came from the mass spectra of the acetates, where, although molecular ions could not be detected in either case, there were ions at *m/e* 738 formed by ready loss of keten. In addition to the

[4] Ch. B. Rao, N. K. Murty, R. V. L. Rao, and N. Venkateswarlu, *Rec. Trav. chim.*, 1964, **83**, 1122.

[5] R. U. Lemieux, R. K. Kullnig, H. J. Bernstein, and W. G. Schneider, *J. Amer. Chem. Soc.*, 1958, **80**, 6098; F. W. Lichtenthaler and P. Emig, *Tetrahedron Letters*, 1967, 583, and references therein.

deacetylated aglycone at *m/e* 192, both showed fragments at *m/e* 547 ($C_{23}H_{31}O_5$) and were readily attributed to


(*a*) *m/e* 331


(*b*) *m/e* 259

oxonium ions from an acetylated pentosylhexosyl unit.[6] Furthermore, both had intense *m/e* 259 (*b*) and relatively weak *m/e* 331 (*a*) peaks, corresponding to terminal pentosyl rather than hexosyl units. Treatment of either glycoside with β-glucosidase released the aglycone (1a), presumably indicating a β-glucosidic linkage to the C-7 phenolic group. Hence both disaccharides must have the part structure (1c) and differ only in the identity of the pentose or in its mode of attachment to the glucose.

## EXPERIMENTAL

Physical and spectral data were obtained as follows: m.p.s on a Kofler hot-stage apparatus; optical rotations on an ETL–NPL automatic polarimeter; u.v. spectra in methanol on a Unicam SP 800 spectrometer; i.r. spectra on a Perkin-Elmer 257 spectrometer; n.mr. spectra on a Varian HA-100 instrument. T.l.c. was performed on Merck silica $F_{254}$ plates and spots were located by u.v. illumination and by spraying with a saturated solution of cerium(IV) sulphate in 6N-sulphuric acid. Spectroscopic data are available as Supplementary Publication No. SUP 21425 (6 pp.).*

*Extraction of* A. rubescens *Bark*.—Finely powdered, air-dried bark (1 kg) was extracted with methanol (20 l) and the extract was evaporated under reduced pressure to leave an orange powder (210 g). This was dissolved in aqueous methanol (10 : 1; 2.2 l) and extracted first with chloroform (5 l) and then with ethyl acetate (5 l); emulsions were broken by centrifugation. Removal of the ethyl acetate under reduced pressure left a powder (52 g) which was further fractionated by gel-permeation chromatography (in five batches) on Sephadex LH-20 (600 g) (methanol as eluant; monitoring by u.v. and t.l.c.). Part (0.7 g) of one fraction (4.2 g) was chromatographed on silica with ethyl acetate to yield 5,7-*dihydroxy*-2-*methylchromone* (1a), thick needles (28 mg), m.p. 275—280° (with sublimation) (from methanol) (Found: $M^+$, 192.0427. $C_{10}H_8O_4$ requires *M*, 192.0423).

The aqueous phase was diluted with methanol (2 l), extracted again with chloroform (2.5 l), filtered to remove some tarry material, and freeze-dried after removal of methanol to give a buff powder (110 g). Part (3.5 g) of this was chromatographed on Sephadex LH-20 (600 g) in methanol as above, and fractions with chromone u.v. absorption were combined and evaporated to leave a hygroscopic residue (0.5 g). Acetylation of this material (60 mg) with acetic anhydride–pyridine afforded two products, as indicated by silica with 2 : 1 ether–ethyl acetate (two developments), both giving an olive-green colour after spraying with cerium(IV) sulphate–sulphuric acid and warming. Separation by t.l.c. gave the 5-hydroxy-2-methyl-7-pentosylglucosyloxychromone hepta-acetates (1i) as amorphous powders: (i) $R_F$ 0.52 (29 mg), $[\alpha]_D^{25}$ −52° (MeOH); (ii) $R_F$ 0.25 (10 mg), $[\alpha]_D^{25}$ −57° (MeOH) (Found: $M^+$, 547.1672. Calc. for $C_{23}H_{31}O_{15}$. *M*, 547.1663).

*Extraction of* A. rubescens *Leaf*.—Freshly gathered leaves (160 g) were homogenised in a blender with methanol (600 ml), and the solid residue was filtered off and further extracted with methanol (3 × 600 ml). The combined extracts were concentrated under reduced pressure and, after extraction with ether to remove non-polar material, the largely aqueous solution deposited a solid (1.2 g). Several recrystallisations from methanol gave 7-*glucosyloxy*-5-*hydroxy*-2-*methylchromone* (1b) as white needles, m.p. 245—248° $[\alpha]_D^{25}$ −50° (pyridine) (Found: C, 54.2; H, 5.3%; $M^+$, 354.0968. $C_{16}H_{18}O_9$ requires C, 54.2; H, 5.1%; *M*, 354.0951).

Acetylation with acetic anhydride–pyridine on a steam-bath for 3 h gave the penta-acetate (1g), needles, m.p. 124—129° (from methanol), $[\alpha]_D^{25}$ −35° (MeOH); $R_F$ 0.56 ($Et_2O$–EtOAc, 2 : 1 × 2). Brief acetylation at room temperature for 30 min gave the amorphous *tetra-acetate* (1h), $[\alpha]_D^{25}$ −37° (MeOH), $R_F$ 0.78 (Found: $M^+$, 354.0968. $C_{16}H_{18}O_{19}$ requires *M*, 354.0951).

*Hydrogenation of* 5-*Acetoxy*-7-*tetra*-O-*acetylglucosyloxy*-2-*methylchromone* (1g).—The penta-acetate (3 mg) in methanol (2 ml) was hydrogenated over Adams catalyst (1 mg) for 75 min. After removal of the catalyst and solvent the residue was purified by t.l.c. to afford 5-acetoxy-7-tetra-*O*-acetylglucosyloxy-2-methylchroman (4).

7-*Acetoxy*-5-*methoxy*-2-*methylchromone* (1f).—The chromone glucoside (1b) (5 mg) in methanol (10 ml) was treated with an excess of ethereal diazomethane at 5 °C for 24 h. After addition of a few drops of acetic acid, evaporation under reduced pressure gave the 5-methyl ether (1d) as a glass. Heating with 3% hydrochloric acid in aqueous methanol (1 : 1; 14 ml) on a steam-bath for 30 min, removal of solvents *in vacuo*, and t.l.c. (silica; benzene–ethyl acetate–methanol, 2 : 2 : 1) afforded the aglycone (1e) as a glass (1 mg). Acetylation in the usual manner and t.l.c. (silica; benzene–ethyl acetate, 1 : 1) gave 7-acetoxy-5-methoxy-2-methylchromone (1f).

*Enzymic Cleavage of the Glycosides* (1b *and* c).—The glycoside (10 mg) was dissolved in pH 5.0 citrate–phosphate buffer (5 ml), β-glucosidase (5 mg) was added, and the solution was left under nitrogen at 37 °C. After 36 h, extraction with ethyl acetate (10 ml) afforded the aglycone, identical with the 5,7-dihydroxy-2-methylchromone (1a) previously isolated.

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* For details of Supplementary Publications, see Notice to Authors No. 7, *J.C.S. Perkin I*, 1974, Index issue.

[6] K. Biemann, D. C. De Jongh, and H. K. Schnoes, *J. Amer. Chem. Soc.*, 1963, **85**, 1763.