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C-ARABINOSYLAPIGENIN METHYL ETHERS FROM *ASTEROSTIGMA RIEDELIANUM*

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Key Word Index *Asterostigma riedelianum*; Araceae; 6,8-di-*C*-arabinosylapigenin 7,4′-dimethyl ether; 2″-*O*-glucosyl-6-*C*-arabinosylapigenin 7,4′-dimethyl ether (and 7-monomethyl ether); 2″-*O*-(caffeoyl)glucosyl-6-*C*-arabinosylapigenin 7,4′-dimethyl ether (and 7-monomethyl ether).

Abstract The leaves of summer harvested *Asterostigma riedelianum* were found to contain the following flavonoids all of which are reported for the first time: 6,8-di-*C*-arabinosylapigenin 7,4′-dimethyl ether, 2″-*O*-glucosyl-6-*C*-arabinosylapigenin 7,4′-dimethyl ether and 2″-*O*-(caffeoyl)glucosyl-6-*C*-arabinosylapigenin 7,4′-dimethyl ether. Winter harvested *A. riedelianum* additionally contained the 7-monomethyl ethers of the mono-*C*-arabinosides.

During the course of a flavonoid survey of the family Araceae [1], three unusual constituents were discovered in leaves of the species *Asterostigma riedelianum* (Schott) O. Kuntze. We now report the full characterization of these compounds.

Two-dimensional paper chromatography of an aqueous methanolic leaf extract of summer harvested *A. riedelianum* revealed the presence of three major flavonoid glycosides: AR-0, AR-1 and AR-2, all appearing as purple spots in UV and unaffected by NH_3. These glycosides were purified by repeated 1D-PC in 15% HOAc and BAW. All three compounds gave similar apigenin-like UV–visible absorption spectra and the reactions with NaOAc and NaOMe shift reagents [2] indicated that each compound had substituents on the 7- and 4′-hydroxyls. Prolonged acid treatment failed to produce an aglycone from any of the glycosides suggesting that they are all 7,4′-disubstituted apigenin *C*-glycosides.

AR-0 (R_f 0.24, BAW and 0.40, HOAc), on acid treatment, isomerized reversibly to an isomer which possessed a lower R_f in HOAc but the same R_f in BAW, behaviour which is analogous with that of apigenin 6,8-di-*C*-arabinoside [3]. Indeed MS of its permethyl (PM) ether, which gave a molecular ion at *m/e* 660, proved AR-0 to be an apigenin di-*C*-pentoside and further, defined the 7- and 4′-substituents as methyl groups. The sugar at C-6 is identified as arabinopyranose since M − 131 > M − 119 > M − 145 [4]. That the second pentose is also arabinose is evidenced by the chromatographic identity of the PM-ether of AR-0 with that of authentic 6,8-di-*C*-α-L-arabinopyranosylapigenin (HL-2) from *Hymenophyton leptopodum* [3, 4] and by the conversion of HL-2 to AR-0 by treatment with CH_2N_2. AR-0 is therefore assigned the structure 6,8-di-*C*-α-L-arabinopyranosylapigenin-7,4′-dimethyl ether.

AR-2 (R_f 0.46, BAW; 0.78, HOAc; 0.53, BEW and 0.96, PhOH) was confirmed as an apigenin derivative by treatment with HI/phenol. Prolonged acid hydrolysis of AR-2 gave a mixture of two isomeric *C*-glycosides with the liberation of glucose. The major, higher R_f isomer (R_f 0.66, BAW; 0.52, HOAc; 0.95, PhOH; 0.69, BEW and 0.83, 50% HOAc) possessed spectral characteristics identical with those of AR-2, thus indicating that the glucose moiety had been attached to the *C*-linked sugar. MS of the PM-ether of this isomer revealed M^+ at *m/e* 486 and the parent ion at *m/e* 455, so defining the isomer as a 6-*C*-pentosylapigenin 7,4′-dimethyl ether. The MS fragmentation pattern and ion intensities matched closely those reported for isomollupentin (6-*C*-α-L-arabinopyranosylapigenin) [5] and indeed the PM-ether proved to be chromatographically identical with the PM-ether of authentic isomollupentin. The major isomer is thus defined as isomollupentin 7,4′-dimethyl ether. The minor isomer, R_fs: 0.50 (BAW) and 0.22 (HOAc), was not present in sufficient amount to allow further investigation. AR-2 was confirmed as a mono-*O*-glucoside of the former by the MS of its PM-ether in which M^+ appeared at *m/e* 690. Major ions 'SO' at *m/e* 471 and 'S' at *m/e* 455 (see Scheme 1) represent fragments that have lost the glucose moiety and which can only be derived from a 2″-*O*-linked glucose [6, 7]. AR-2 is accordingly formulated as 2″-*O*-glucosyl-6-*C*-α-L-arabinopyranosylapigenin 7,4′-dimethyl ether (**1**) (2″-*O*-glucosylisomollupentin 7,4′-dimethyl ether).

AR-1 (R_f 0.51, BAW; 0.60 HOAc; 0.57, BEW and 0.97 in PhOH) on prolonged acid treatment gave glucose plus the same two isomers produced from AR-2. Paper chromatography also revealed conversion of AR-1 to AR-2, some unchanged AR-1 and two pairs of blue fluorescent compounds with R_fs approximating to those of *cis*/*trans*-cinnamic acids and the corresponding glycosyl derivatives. Alkaline treatment of AR-1 gave total conversion to AR-2 with the liberation of caffeic acid, which was identified by TLC comparison with an authentic marker. MS of the PM-ether of AR-1 did not give a detectable molecular ion but did confirm the presence of a 2″-*O*-linked glucose. Further, the presence of M − 175, 'SO' and 'S' ions in abundances comparable with those produced by AR-2 indicates that

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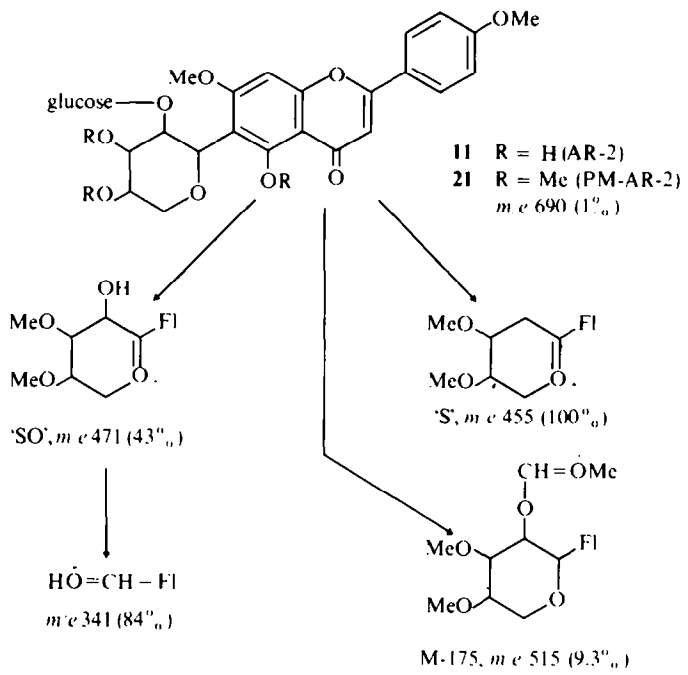

Scheme 1. Key fragmentations observed in the mass spectrum of permethylated AR-2.

the caffeoyl moiety is not attached to the arabinose. Accordingly AR-1 is assigned the structure 2″-*O*-(caffeoyl)glucosyl-6-*C*-α-L-arabinopyranosylapigenin 7,4′-dimethyl ether.

Leaves of *Asterostigma riedelianum* collected in winter gave the same 2D-PC flavonoid pattern as the material collected in summer except that the lower portions of both the AR-1 and AR-2 spots turned yellow in NH_3 vapour. Acid treatment of these samples produced isomeric products equivalent to those from AR-1 and AR-2 but which also contained dark to yellow (UV + NH_3) components R_fs: 0.46, 0.59 (BAW) and 0.20, 0.44 (HOAc), respectively. UV–visible absorption data for the purified dark to yellow contaminants indicated that in both cases the 7-hydroxyls were methylated and that the 4′-hydroxyls were free, suggesting that the winter harvested plant had incompletely methylated substances present. This relationship was confirmed in the case of the contaminant in the major hydrolysis product by treatment with CH_2N_2 which produced isomollupentin 7,4′-dimethyl ether quantitatively.

The 7- and 7,4′-dimethyl ethers of isomollupentin, 2″-*O*-glucosylisomollupentin and 2″-*O*-(caffeoyl)glucosylisomollupentin are all reported for the first time.

EXPERIMENTAL

Fresh leaf material of *Asterostigma riedelianum* (Accession No. 106-77.00963) was collected from the Royal Botanic Gardens, Kew and a voucher specimen is deposited in the herbarium (K).

Identification of leaf flavonoids. 2D-PC was carried out on Whatman No. 1 or 3MM paper using *n*-BuOH–HOAc–H_2O, 4:1:5 organic layer (BAW) and 15% HOAc (HOAc). Other solvents used include: *n*-BuOH–EtOH–H_2O, 4:1:2 (BEW) and PhOH–H_2O, 4:1 (PhOH). UV-visible absorption spectra were recorded using shift reagents as described in [2]. MS were obtained by direct insertion of samples into the ion source.

Isolation of flavonoids. Leaves of *A. riedelianum* were macerated with MeOH–H_2O (3:1) and left overnight to extract. The concd extract was run in HOAc on 3MM paper yielding bands of AR-0, AR-1 and AR-2, which were further purified by rechromatography in BAW.

Hydrolysis conditions. (a) Prolonged acid hydrolysis: the sample dissolved in MeOH–2 N HCl (1:1) was refluxed for 1.75 hr. (b) Alkaline hydrolysis: the sample in a minimum of H_2O was treated with 1 N NaOH under N_2 in a Thunberg tube for 2 hr.

Derivatization techniques. (a) Methylation: the sample in MeOH was treated with CH_2N_2 in Et_2O. After 10 min further CH_2N_2 was added and the product worked up after 10 min standing. (b) Permethylation: the sample (1 mg) in dry DMF was added to petrol-washed NaH powder under N_2 in a septum-sealed container. To this was added MeI (0.2 ml) by syringe and the mixture allowed to stand in the dark for 1 hr. After addition of MeOH and H_2O, the permethyl ether was isolated by $CHCl_3$ extraction followed by TLC (Si gel, Me_2CO–$CHCl_3$, 1:4).

Sugar analyses. Sugars were identified by PC against standard markers in BBPW (*n*-BuOH–C_6H_6–pyridine–H_2O, 5:1:3:3 top layer), BAW, BEW and PhOH.

Physical data. AR-0. λ_{max} (MeOH) 273, 335 nm; (NaOMe) 286 inc, 295 sh, 384 dec. nm; (NaOAc) 273, 335 nm. MS (*m/e*, PM ether): 660 (16.4%, M^+), 659 (15.3%), 645 (22.8%), 629 (100%, M – 31), 541 (20.6%, M – 119), 529 (31.5%, M – 131), 515 (15.3%, M – 145), etc.

AR-1. λ_{max} (MeOH) 273, 333 nm; (NaOMe) 285 inc, 295 sh, 387 dec. nm; (NaOAc) 273, 334 nm. MS (*m/e*, PM ether): 515 (5.7%), 501 (4.8%), 472 (9.0%, SO + H), 471 (39.7%, SO), 456 (29.4%, S + H), 455 (100%, S), 341 (82.2%, Fl–CH=OH^+), 325 (10.6%, Fl–CH_2^+), etc.

AR-1 (component turning yellow in NH_3*).* λ_{max} (MeOH) 273, 335 nm; (NaOMe) 287, 395 inc. nm; (NaOAc) 273, 331 nm.

AR-2. λ_{max} (MeOH) 274, 332 nm; (NaOMe) 290 inc, 385 dec. nm; (NaOAc) 274, 332 nm. MS (*m/e*, PM ether): 690 (1.0%, M^+), 515 (9.3%, M – 175), 501 (6.8%), 472 (13.1%, SO + H), 471

(43.3%, SO), 456 (33.2%, S + H), 455 (100%, S), 341 (83.9%, Fl—CH=OH$^+$), 325 (12.3%, Fl—CH$_2^+$), etc.

Conditions for co-chromatography. (a) *Permethyl ethers*, TLC, Si gel using Me_2CO–$CHCl_3$ (1:4), $CHCl_3$–EtOAc–Me_2CO (5:4:1) and EtOAc-pyridine–H_2O–MeOH (16:4:2:1). (b) *Flavone C-arabinosides*, PC using BAW, HOAc, H_2O and $CHCl_3$–HOAc–H_2O (30:15:2).

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ACYLATED FLAVONE-*C*-GLYCOSIDES FROM THE SEEDS OF *ZIZYPHUS JUJUBA**

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Key Word Index—*Zizyphus jujuba*; Rhamnaceae; acylated flavone-*C*-glycoside; 6‴-sinapoylspinosin; 6‴-feruloylspinosin; 6‴-*p*-coumaroylspinosin.

In a previous paper [1], we reported the isolation and structure elucidation of a new flavone-*C*-glycoside, spinosin, from the seeds of *Zizyphus jujuba* (*Z. vulgaris* var. *spinosus*) which have long been used in traditional medicine for treating insomnia and nervous debility. This communication deals with the chemistry of new acylated spinosins. Repeated column chromatography of the ethyl acetate-soluble fraction of the MeOH extract of the seeds on silica gel yielded an acylated spinosin mixture. This was further separated using another solvent system into three components **1**–**3** in order of decreasing polarity.

1. $C_{39}H_{42}O_{19}\cdot 2H_2O$, mp 198–204, $[\alpha]_D^{20} - 40.5°$ (MeOH), gave characteristic flavonoid colour reactions and a positive Molisch test. IR showed OH, α,β-unsaturated ester and carbonyl absorptions at 3350, 1690 and 1650 cm^{-1} respectively and a broad C—O stretching band in the region 1100–1000 cm^{-1}, suggesting its glycosidic nature. Acid hydrolysis of **1** gave swertisin, mp 242–244° (mmp, co-TLC), glucose (TLC and GLC as TMSi ether) and sinapic acid (GLC as TMSi ether), while mild alkaline hydrolysis yielded spinosin (**4**), mp 255–256° (mmp and co-TLC). The UV absorption of **1** and the bathochromic shifts with diagnostic reagents [2] suggested that the acyl residue must be attached to one of sugar OH groups.

Acetylation gave a nonaacetate, mp 124–128°, showing six sugar acetate methyl signals, three phenolic acetate methyl signals and three oxymethyl signals in its ^{1}H NMR spectrum. This observation indicated that **1** was composed of 1 mol of **4** and 1 mol of sinapic acid. Acetone treatment yielded a monoisopropylidene derivative. Permethylated **1** and permethylated monoisopropylidene derivative showed in the mass spectra intense peaks at *m/e* 499 and 515, and 511 and 527, respectively, corresponding to the loss of

* Part 18 in the series "Structure of Flavone-*C*-glycosides". For Part 17 see [1].