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(−)-EPIAFZELECHIN 5-*O*-β-D-GLUCOSIDE FROM *CRATAEVA RELIGIOSA*

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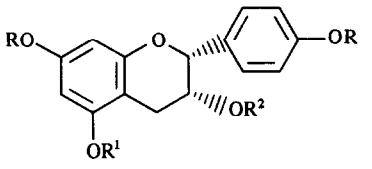
Key Word Index—*Crataeva religiosa*, Capparidaceae, flavan-3-ols, (−)-epiafzelechin 5-*O*-β-D-glucoside

Abstract—Epiafzelechin 5-glucoside was characterized from the bark of *Crataeva religiosa* together with other known compounds

Crataeva religiosa Forst f is well known for its medicinal uses [1] Earlier chemical work on this plant is confined to triterpenoids and flavanoids [2–4] In the present paper we report the isolation and characterization of flavan-3-ols and a glucoside from the alcoholic extract of this plant Flavan-3-ol glucosides are of very rare occurrence in the plant kingdom and mostly occur as gallates [5] Previous reports of such glycosides are restricted to a few flavan-3-ols [6] The high percentage of (−)-epiafzelechin 5-glucoside in *C religiosa* is most unusual

The defatted alcoholic extract of the plant after concentration and solvent extraction with ethyl acetate resulted in the separation of four compounds after column chromatography over silica gel Three of these were characterized as sitosterol glucoside, (−)-epiafzelechin and (−)-catechin The last and the most polar of the four compounds which is being reported for the first time was crystallized from ethyl acetate and was identified as (−)-epiafzelechin 5-*O*-β-D-glucoside (**1**) in the following manner Elemental analysis corresponded to the molecular formula $C_{21}H_{24}O_{10}$ IR showed very strong absorption at 3500 cm^{-1} and no absorption for a carbonyl function Its UV spectrum in methanol showed maxima at 278 and 315 nm characteristic of a flavone system No UV shift with $AlCl_3$ indicated the absence of an *ortho*-dihydroxy grouping In the ^1H NMR spectrum of the compound in DMSO-d_6 two doublets at δ7 40 and 6 83 (J = 8 5 Hz) were assigned to the protons of an A_2B_2 system in ring B Two *meta* coupled doublets at δ6 10 and 6 23 (J = 2 5 Hz) are due to C-6 and C-8 protons and C-4 protons signals were observed in the form of an envelope at δ2 90 Acetylation of **1** gave a hepta-acetate **1a** Acetate signals were visible at δ1 90, 2 0 and 2 30 for C-3 acetate, sugar acetoxyls and aromatic acetate groups, respectively The signals for A_2B_2 protons shifted to δ7 03 and 7 40, respectively The C-6 and C-8 protons were located at δ6 36 and 6 46 These two doublets however, merged to become a two proton singlet when the NMR spectrum was run in CCl_4 with a few drops of $CDCl_3$ added to fully solubilize the substance

Hydrolysis of **1** with emulsin gave glucose and an aglycone **1b** In the ^1H NMR spectrum of **1b** in DMSO-d_6, A_2B_2 proton signals were observed at δ6 93 and 7 30 and signals for two *meta* coupled protons of ring A were located at δ5 83 and 5 96 Also, a weakly coupled singlet at δ4 96 was assigned to the C-2 proton Acetylation of **1b** gave a tetra-acetate **1c** In the ^1H NMR spectrum of **1c** three extra signals were visible at δ2 80 for two protons at C-4, 5 03 and 5 30 each integrating for a single proton assignable to the C-2 and C-3 protons, respectively The weak coupling between these two is indicative of the *cis* nature of these protons Compounds **1b** and **1c** were confirmed as epiafzelechin and its acetate by melting point, specific rotation and spectral data The shift of C-6 and C-8 protons to δ6 43 and 6 63 and A_2B_2 protons of the B ring to δ7 0 and 7 30 clearly shows glucosidation in the A ring



1 R = R^2 = H
R^1 = Glc
1a R = R^2 = COMe
R^1 = Glc Tetra Acetate
1b R = R^1 = R^2 = H
1c R = R^1 = R^2 = COMe
1d R = Me, R^1 = R^2 = H
1e R = Me, R^1 = R^2 = COMe

Table 1 ^{13}C NMR of (−)-epiafzelechin 5-glucoside

C-2	81 2	C-2′	127 8
C-3	64 7	C-3′	114 5
C-4	27 9	C-4′	154 9
C-4a	99 5	C-5′	114 5
C-5	156 5	C-6′	127 8
C-6	96 0	C-1″	100 7
C-7	156 1	C-2″	73 8
C-8	96 9	C-3″	76 8
C-8a	156 1	C-4″	70 1
		C-5″	78 3
C-1′	129 5	C-6″	61 2

Methylation of **1** and subsequent hydrolysis of the methylated product gave **1d** The ^{1}H NMR spectrum of **1d** in $CDCl_3$ displayed signals for C-3 and C-2 protons at δ4 26 (*br s*) and δ4 96 (s), respectively A_2B_2 proton signals were located at δ6 90 and 7 40 The C-6 and C-8 proton signals were observed at δ6 03 and 6 13, respectively and signals for C-4 protons were distinguishable at δ2 96 Acetylation of **1d** gave **1e** In the ^{1}H NMR spectrum ($CDCl_3$) of **1e** signals for C-6 and C-8 protons registered a shift to δ6 30 and 6 36, respectively The other signals remained almost at their original positions This observation is also indicative of the glucosidation in the A ring

The position of glucose in the A ring was finally established at C-5 from the Gibb's test of **1** and **1d** which was positive in the latter case The position was also corroborated from the comparative study of C-6 and C-8 protons shifts in the ^{1}H NMR spectra of **1**, **1a** and **1e**

EXPERIMENTAL

All the mps are uncorr The ^{1}H NMR spectra were recorded on a Varian T-60A and ^{13}C NMR on a JEOL-90 with TMS as int standard The plant material was procured from the Bombay region (R R L Herbarium accession number 1074) and the bark was extracted with petrol and then EtOH The conc EtOH extract was subjected to partition with C_6H_6, EtOAc and *n*-BuOH, respectively The EtOAc extract was concd, charged over a silica gel column and eluted with various proportions of $CHCl_3$ and MeOH Four pure compounds were obtained in this manner The first three were identified as sitosterol glucoside, (−)-epiafzelechin and (−)-catechin by co-TLC, superimposable IR and comparison of ^{1}H NMR spectra with authentic samples The fourth and major component **1** was crystallized from EtOAc as buff coloured crystals, mp 190°, $[\alpha]_D^{MeOH}$ −38 3° It analysed for $C_{21}H_{24}O_{10}$ (Found C, 57 76, H, 5 61%, calc C, 57 8, H, 5 5%) ^{1}H NMR (DMSO-d_6) δ2 90 (envelope 2H, H-4), 6 10 (1H, *d*, *J* = 2 5 Hz, H-6), 6 23 (1H, *d*, *J* = 2 5 Hz, H-8), 6 83 (2H, *d*, *J* = 8 5 Hz, H-3′ and H-5′), 7 40 (2H, *d*, *J* = 8 5 Hz, H-2′ and H-4′) MS of **1** showed fragments at *m/z* 436 $[M]^+$, 396, 308, 274, 255, 254, 211, 139, 136, 98, 70, 61 (100%) and 44 IR ν_{max}^{KBr} cm^{-1} 3400, 1610, 1530, 1500, 1450, 1370, 1220, 1110, 1050

Acetylation of **1** Acetylation (Ac_2O–pyridine) gave a heptaacetate **1a** crystallized from MeOH, mp 210° ^{1}H NMR ($CDCl_3$) δ1 90 (3H, *s*, OAc), 2 00 (12H, 4 × OAc), 2 30 (3H, *s*, 2 × ArOAc), 2 80 (2H, envelope, H-4), 3 76 (*br m*, –CH, sugar), 4 16 (2H, envelope C<u>H</u>$_2$OAc sugar), 5 0–5 40 (6H, *m*, sugar and H-3 and H-4), 6 36 and 6 46 (2 × 1H, *d*, *J* = 2 5 Hz, H-6, H-8), 7 03 (2H, *d*, *J* = 8 5 Hz, H-3′, H-5′), 7 40 (2H, *d*, *J* = 8 5 Hz, H-2′, H-6′)

Enzyme hydrolysis of **1** Compound **1** (100 mg) was dissolved in EtOH and emulsin added The mixture was kept at room temp for 24 hr and processed as usual The sugar was identified as glucose by PC The aglycone **1b** was extracted with EtOAc and crystallized from the same solvent mp 246°, $[\alpha]_D^{MeOH}$ −63° It analysed for $C_{15}H_{14}O_5$ (Found C, 65 75, H, 5 04, Calc C, 65 69, H, 5 11%) ^{1}H NMR of **1b** (DMSO-d_6) δ2 80 (2H, envelope, H-4), 4 96 (1H, *s*, H-2), 5 83 and 5 96 (2 × 1H, *d*, *J* = 2 5 Hz, H-6, H-8), 6 93 (2H, *d*, *J* = 8 5 Hz, H-3′, H-5′), 7 30 (2H, *d*, *J* = 8 5 Hz, H-2′, H-6′) MS of **1b** showed fragments at *m/z* 274 $[M]^+$, 211, 167, 139 (100%), 136, 107, 51, 43 Acetylation of **1b** in pyridine and Ac_2O resulted in the formation of a tetra-acetate **1c** crystallized from MeOH, mp 125–126° ^{1}H NMR of **1c** ($CDCl_3$) δ1 83 (3H, *s*, OAc), 2 26 (9H, *s*, 3 × Ar–OAc), 2 80 (2H, envelope, H-4), 5 03 (1H, *s*, H-2), 5 30 (1H, *m*, H-3), 6 43 (1H, *d*, *J* = 2 5 Hz, H-6), 6 63 (1H, *d*, *J* = 2 5 Hz, H-8), 7 00 (2H, *d*, *J* = 8 5 Hz, H-3′, H-5′) and 7 30 (2H, *d*, *J* = 8 5 Hz, H-2′, H-6′)

Methylation of **1** with CH_2N_2 in MeOH gave a methyl ether which was hydrolysed with emulsin to give **1d**, an amorphous powder ^{1}H NMR of **1d** ($CDCl_3$) δ2 96 (2H, envelope, H-4), 3 76 (3H, *s*, Ar–OMe), 3 86 (3H, *s*, Ar–OMe), 4 26 (1H, *br s* H-3), 4 96 (1H, *s*, H-2), 6 03 and 6 13 (2 × 1H, *d*, *J* = 2 5 Hz, H-6, H-8), 6 93 (2H, *d*, *J* = 8 5 Hz, H-3′, H-5′), 7 50 (2H, *d*, *J* = 8 5 Hz, H-2′, H-6′) Acetylation of **1d** (pyridine–Ac_2O) gave a diacetate **1e**, an amorphous powder ^{1}H NMR of **1e** ($CDCl_3$) δ1 80 (3H, *s*, OAc), 2 20 (3H, *s*, Ar–OAc), 2 76 (2H, envelope, H-4), 3 70 (3H, *s*, Ar–OMe), 3 73 (3H, *s*, Ar–OMe), 5 00 (1H, *s*, H-2), 5 26 (1H, *m*, H-3), 6 30 and 6 36 (2 × 1H, *d*, *J* = 2 5 Hz, H-6, H-8), 6 80 (2H, *d*, *J* = 8 5 Hz, H-3′, H-5′), 7 26 (2H, *d*, *J* = 8 5 Hz, H-2′, H-6′)

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