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FLAVANS FROM *ZEPHYRANTHES FLAVA**

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Key Word Index—*Zephyranthes flava*; Amaryllidaceae; 7-hydroxy-3',4'-methylenedioxyflavan, 7,4'-dihydroxy-3'-methoxyflavan; 7-methoxy-2'-hydroxy-4',5'-methylenedioxyflavan; 7-glucosyloxy-3',4'-methylenedioxyflavan.

Abstract—A new optically active flavan aglucone, 7-hydroxy-3',4'-methylenedioxyflavan, and its 7-glucoside have been isolated from the bulbs of *Zephyranthes flava*, collected at flowering. Additionally, two known flavans, 7,4'-dihydroxy-3'-methoxyflavan and 7-methoxy-2'-hydroxy-4',5'-methylenedioxyflavan, have been isolated for the first time from this species. The structures of these flavans have been established by comprehensive analyses (UV, IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry, $[\alpha]_D$) of the compounds and their acetates, and also by chemical correlation.

INTRODUCTION

Zephyranthes flava Roem. & Schult. (Amaryllidaceae) is a deciduous herb native to America and has now been naturalized in India. The 15–20 cm plant grows abundantly in the upper Gangetic plains and bears yellow flowers during the rainy season (July–August). The extract of its bulbs is used in the indigenous system of medicine for a variety of therapeutic purposes, e.g. in the treatment of diabetes, for ear and chest ailments, and against viral infections. The bulbs contain a complex mixture of alkaloids [2, 3] and non-nitrogenous aromatic compounds. The present paper describes the isolation and characterization of four flavans [4] from the bulbs of this species.

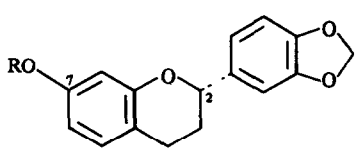
RESULTS AND DISCUSSION

From the petrol and ethanol extracts of bulbs of *Z. flava*, collected at the first onset of flowers, three flavan aglucones (**1**–**3**) and a glucosyloxyflavan (**4**) were isolated. Among these compounds, two are new flavans (compounds **1** and **4**) and two (**2** and **3**) were reported earlier from other plant families [5, 6] but encountered for the first time in the title species. Characterization of the new flavans only is described here.

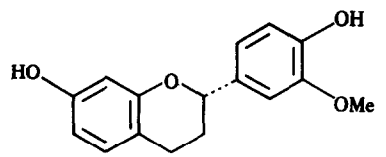
Compound **1**, $C_{16}H_{14}O_4$ (by accurate mass measurement), $[\alpha]_D$ −14.2° ($CHCl_3$), exhibited UV and IR spectra typical of naturally occurring flavans [4]. The mass spectra of the compound and its acetate derivative were also characteristic of those of flavans [4]. Thus, the fragment-ion peak, of appreciable abundance, at *m/z* 123 was due to the retro-Diels–Alder reaction of the compound with concomitant H transfer into the fragment ion (**5**). The chemical shifts, coupling patterns and constants in the ^{1}H NMR and ^{13}C NMR spectra of the compound were consistent with the 7-hydroxy-3',4'-methylenedioxy structure (**1**) assigned to it.

Compound **4**, $C_{22}H_{24}O_9 \cdot H_2O$ (by combustion analyses and accurate mass measurement of its tetraacetate), $[\alpha]_D$ −37.2° (MeOH), was obtained as a hygroscopic solid. It showed a negative ferric reaction. The UV

*Part 10 in the series "Chemical Constituents of Amaryllidaceae". For Part 9 see ref. [1].



1 R = H
4 R = Glc



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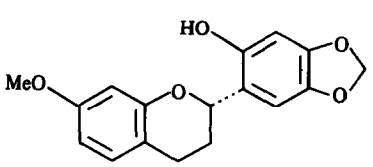

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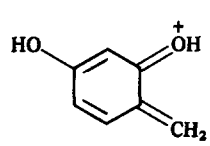

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maxima of the compound were characteristic of a *para*-substituted alkoxybenzene. It afforded a tetraacetate, the ^{1}H NMR spectrum (chemical shifts, coupling patterns and constants) of which suggested a 7-glycosylated 3',4'-methylenedioxyflavan structure. Hydrolysis of the compound with emulsin gave **1** as the aglucone and D-glucose. On the basis of these data, the 7-glucosyloxy-3',4'-methylenedioxyflavan structure (**4**) was assigned to this compound. This flavan has not been encountered before in nature nor has it previously been prepared synthetically.

The stereochemistry of the flavans at C-2 (**1** and **4**) was established on the basis of the sign of their optical rotation and the splitting pattern [7] of H-2 in their respective ^{1}H NMR spectra.

7-Glucosyloxy-3',4'-methylenedioxyflavan (**4**) is the fifth example of a naturally occurring glucosyloxyflavan (devoid of any oxygen substituent at C-3, C-4 of the heterocyclic ring). Furthermore, this is the first report of flavans occurring naturally in the Amaryllidaceae, although such substances have been described as phytoalexins in *Narcissus* [8].

EXPERIMENTAL

The general procedures were the same as those reported recently [9].

Isolation procedure. The plant materials were collected from the Banaras Hindu University Campus during July 1982 and were identified by Professor S. K. Roy, Department of Botany, Banaras Hindu University. The species is now being cultivated in the medicinal garden of the Department of Pharmaceutics, Banaras Hindu University, Varanasi. Dried and milled bulbs of *Z. flava* (*ca* 0.5 kg) were successively extracted in a Soxhlet with petrol (60–80°) (fraction A) and EtOH (fraction B), 30 hr each, and the two fractions were separately processed.

Treatment of fraction A. The petrol concentrate was passed through a column of alumina (Brockman neutral, activity grade IV, 24 × 2 cm). Elution was carried out with petrol (1 l.), C_6H_6 (1.2 l.), and C_6H_6–$CHCl_3$ (1.5 l.). Fractions (100 ml) were collected and monitored by analytical TLC.

7-Methoxy-2'-hydroxy-4',5'-methylenedioxyflavan (**3**). The middle C_6H_6–$CHCl_3$ eluates were combined and concd to give **3** which crystallized from C_6H_6 as colourlss needles (43 mg), mp 163–165°; UV λ_{max}^{MeOH} nm (log ε): 225 (4.09), 285 (3.82), 288 (3.88), ~ 300 (sh); IR ν_{max}^{KBr} cm^{-1}: 3400, 1610, 1595, 1038, 938; ^{1}H NMR ($CDCl_3$): δ6.9 (1H, *d*, *J* = 8 Hz, H-5), 6.82 (1H, *s*, exchangeable with D_2O, C-2'-OH), 6.55 (1H, *dd*, *J* = 8, 2.5 Hz, H-6), 6.47 (1H, *s*, H-3'), 6.42 (1H, *d*, *J* = 2.5 Hz, H-8), 5.90 (2H, *s*, OCH_2O), 4.9 (1H, *q*, *J* = 8, 4 Hz, H-2), 3.74 (3H, *s*, OMe); MS *m/z* (rel. int.): 300 $[M]^+$ (100), 178 (9), 164 (80), 163 (28), 151 (24), 150 (48), 138 (21), 137 (82), 94 (5) ($[M]^+$ by accurate mass measurement: 300.0989. $C_{17}H_{16}O_5$ requires: M, 300.0993). The physical and spectral properties were indistinghishable from those reported in the literature [5].

Treatment of fraction B. The residue from this fraction was triturated with aq. HOAc (4%, 100 ml); the mixture was kept at room temp. overnight, and filtered. The filtrate and the residue were marked fractions B_1 and B_2, respectively.

Treatment of fraction B_1. This fraction was extracted with Et_2O (3 × 50 ml). The Et_2O-soluble acetates, consisting of weaker bases and non-nitrogenous constituents, were separated by CC over Al_2O_3 (30 g). The aq. acidic mother liquor on basification and usual processing of the liberated bases afforded the major alkaloids [3]. The early C_6H_6–$CHCl_3$ eluates from the Al_2O_3 column afforded a further crop (7 mg) of flavan **3**.

7-Hydroxy-3',4'-methylenedioxyflavan (**1**) The later C_6H_6–$CHCl_3$ eluates from the Al_2O_3 column afforded **1** as colourless micro-crystals (26 mg), mp 122–124°; $[\alpha]_D^{22}$ −14.2° (*c* 0.3; $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 225 (sh) (3.66), 278 (3.62), 282 (sh) (3.60); IR ν_{max}^{KBr} cm^{-1}: 3400, 1612, 1592, 940, 835; ^{1}H NMR ($CDCl_3$): δ7.0 (3H, *m*, H-2', H-5', H-6'), 6.96 (1H, *d*, *J* = 8 Hz, H-5), 6.40 (1H, *dd*, *J* = 8, 2.5 Hz, H-6), 6.32 (1H, *d*, *J* = 2.5 Hz, H-8), 5.90 (2H, *s*, OCH_2O), 5.0 (1H, *s*, exchangeable with D_2O, C_7-OH), 4.92 (1H, *dd*, *J* = 8, 4 Hz, H-2), 2.9–2.6 (2H, *m*, H-4), 2.3–1.9 (2H, *m*, H-3); ^{13}C NMR ($CDCl_3$): δ157.4 (*s*, C-7), 156.6 (*s*, C-9), 148.5 (*s*, C-3'), 148.4 (*s*, C-4'), 135.0 (*s*, C-1'), 130.4 (*d*, C-5), 120.5 (*d*, C-6'), 114.5 (*s*, C-10), 112.0 (*d*, C-5'), 110.3 (*d*, C-2'), 109.8 (*d*, C-6), 104.2 (*d*, C-8), 100.2 (*t*, OCH_2O), 77.5 (*d*, C-2), 30.5 (*t*, C-4), 24.7 (*t*, C-2). The assignments of the ^{13}C NMR signals were made on the basis of literature precedents [7]; MS *m/z* (rel. int.): 270 $[M]^+$ (95), 148 (100), 136 (34), 123 (42), 122 (35), 95 (5), 94 (5) ($[M]^+$ by accurate mass measurements; 270.0884. $C_{16}H_{14}O_4$ requires. $[M]^+$ 270.0888).

7-Acetoxy-3',4'-methylenedioxyflavan, prepared from **1** with Ac_2O and pyridine, was obtained as a thick liquid; ^{1}H NMR ($CDCl_3$): δ7.0 (4H, *m*, H-5, H-2', H-5', H-6'), 6.62 (1H, *dd*, *J* = 8, 3 Hz, H-6), 6.47 (1H, *d*, *J* = 3 Hz, H-8); MS *m/z* (rel. int.): 312 $[M]^+$ (24), 270 (100), 123 (20), 122 (38).

7,4'-Dihydroxy-3'-methoxyflavan (**2**). The $CHCl_3$ eluates from the Al_2O_3 column were combined and concd. The $CHCl_3$ concentrate was subjected to prep. TLC on precoated plates [9] in $CHCl_3$–MeOH (19:1). The R_f zone ~ 0.6 was eluted with $CHCl_3$ and the solvent was evapd to give 7,4'-dihydroxy-3'-methoxyflavan as colourless crystals (22 mg), mp 145–146°; $[\alpha]_D^{22}$ 0° ($CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 225 (sh) (4.1), 282 (3.66); $\lambda_{max}^{MeOH+0.01\,M\,NaOH}$nm: ~ 250, ~ 290; IR ν_{max}^{KBr} cm^{-1}: 3400, 1618, 1610, 1595, 1050; ^{1}H NMR ($CDCl_3$): δ6.90 (1H, *d*, *J* = 8 Hz, H-5), 6.84 (3H, *m*, H-2', H-5', H-6'), 6.40 (1H, *dd*, *J* = 8, 2.5 Hz, H-6), 6.35 (1H, *d*, *J* = 2.5 Hz, H-8), 4.95 (1H, *q*, H-2), 3.9 (3H, *s*, OMe), 2.8 (2H, *m*, H-4), 2.0 (2H, *m*, H-3); MS: *m/z* (rel. int.): 272 $[M]^+$ (100), 150 (90), 137 (82), 135 (78), 123 (30), 122 (25), 94 (7). The physical and spectral properties of the compound were indistinguishable from those reported for **2** in the literature [6].

The 7,4'-diacetate of **2** was prepared by heating with Ac_2O and pyridine on a steam bath (2 hr) followed by usual work-up to afford it as a low melting solid, R_f 0.7 (C_6H_6–$CHCl_3$, 1:1); ^{1}H NMR ($CDCl_3$): δ7.05 (1H, *d*, *J* = 8 Hz, H-5), 6.9 (3H, *m*, H-2', H-5', H-6'), 6.55 (2H, *m*, H-6, H-8), 4.9 (1H, *q*, *J* = 8, 4 Hz, H-2), 2.32 (3H, *s*, OAc), 2.28 (3H, *s*, OAc); MS *m/z* (rel. int.): 356 $[M]^+$ (18), 314 (24), 272 (100), 123 (18).

Treatment of fraction B_2. The brown amorphous solid, obtained from this fraction, was dissolved in MeOH and chromatographed over silica gel (20 × 2 cm). Elution was carried out with C_6H_6 (1 l.), $CHCl_3$ (1 l.), $CHCl_3$–MeOH (99:1, 49:1, 1.5 l. each). Fractions (100 ml) were collected and monitored by analytical TLC using benzidine-metaperiodate (for polyols) as the spraying reagent. The fractions from $CHCl_3$–MeOH (49:1), which showed a major spot for glucoside, were combined and concd to give a light-brown solid (62 mg), mp 140–145° (sealed tube); R_f 0.35 ($CHCl_3$–MeOH, 17:3); $[\alpha]_D^{22}$ −37.2° (*c* 0.51; MeOH), UV λ_{max}^{MeOH} nm (log ε): 222 (sh) (4.0), 270 (3.2), 275 (3.1); IR ν_{max}^{KBr} cm^{-1} 3450 (br), 1615, 1600 (br), 1500, 1070, 938, 830. (Found: C, 58.3; H, 6.0. $C_{22}H_{24}O_9 \cdot H_2O$ requires: C, 58.6; H, 5.7%.)

The tetraacetate, prepared with Ac_2O and pyridine, at room temp. overnight followed by heating on a steam bath (1 hr), crystallized from MeOH as colourless needles, mp 160–163°; IR ν_{max}^{KBr} cm^{-1}: 1750, 1205; ^{1}H NMR: ($CDCl_3$): δ6.94 (3H, *m*, 6.90 (1H, *d*, *J* = 8 Hz, H-5), 6.42 (1H, *dd*, *J* = 8, 2 Hz, H-6), 6.30 (1H, *d*, *J* = 2 Hz, H-8), 5.90 (2H, *s*, OCH_2O), 4.9 (1H, *q*, *J* = 8, 4.5 Hz, H-2), 2.04–2.0 (14 H, H-3, glucosyl OAc); MS *m/z* (rel. int): 600 $[M]^+$ (4), 331 (42), 270 (100).

Enzymatic hydrolysis of glucosyloxyflavan **4**. Glucosyloxyflavan **4** (14 mg) was dissolved in buffer (0.5 M NaOAc, 20 ml, adjusted to pH 5 with HOAc) and was emulsified with emulsin (12 mg) according to ref. [10]. The product, consisting of the aglucone, crystallized from petrol–C_6H_6 as colourless microcrystals. Its identity as 7-hydroxy-3′,4′-methylenedioxyflavan was established by direct comparison (mp, mmp, co-TLC). The identity of the sugar moiety was established as glucose by GC of the corresponding alditol acetate [11].

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