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ANNPHENONE, A PHENOLIC ACETOPHENONE FROM *ARTEMISIA ANNUA**

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Key Word Index—*Artemisia annua*; Compositae; substituted acetophenone; annphenone.

Abstract—Fractionation of the *n*-butanol extract of *Artemisia annua* led to the isolation of a new phenolic acetophenone, the structure of which was elucidated as 2,4-dihydroxy-6-methoxy-acetophenone 4-*O*-β-D-glucopyranoside on the basis of spectroscopic data. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Artemisia annua L. has been the subject of intense chemical investigation over the past decade following the discovery that artemisinin is effective in the treatment of malaria [2, 3]. Although over 40 phenolic compounds have been described from *A. annua* [2–4], there has been no previous report of any substituted acetophenone. Chemical investigation studies on the water-soluble part of an ethanol extract of the aerial parts afforded annphenone (**1**).

RESULTS AND DISCUSSION

Compound **1** was isolated as a crystalline solid, mp 160–62°. IR spectroscopy demonstrated the presence of carbonyl (1640 cm^{-1}) and hydroxyl (3465 cm^{-1}) groups and the ^{13}C NMR/DEPT data for **1** displayed 15 carbon atoms with 15 directly attached protons. The ^{13}C chemical shift values suggested that **1** incorporated three oxygen bearing aryl carbons, two arylmethine carbons and a non-protonated aryl carbon atom, thus identifying the presence of a tetrasubstituted phenyl ring system. The other NMR spectral features included an anomeric signal (^{13}C: δ 99.18; ^{1}H: 4.95, 1H, *d*, $J = 6.9$ Hz), four hydroxymethines (^{13}C: δ 76.35, 75.54, 72.75 and 69.44) and a hydroxymethylene (δ 60.59), suggesting that **1** is a monoglycoside. This led to an inferred composition of $C_{15}H_{22}O_9$ for **1**, which was consistent with $[M]^+$ at *m/z* 344 in the EI mass spectrum and supported by a pseudomolecular ion peak at *m/z* 367 corresponding to $[M + Na]^+$ in its positive-ion FAB mass spectrum. The prominent fragment ion at *m/z* 182 in the EI mass spectrum was consistent with the loss of a hexopyranosyl residue. The ^{1}H NMR spectrum of **1** displayed two methyl singlets at δ 3.69 and 2.34, indicating the presence of a methoxyl and an acetyl group. The ^{13}C signals at δ 55.61 (methyl), 205.72 (C=O) and 32.33 (methyl) supported the above observations. The ^{13}C NMR spectrum also reflected the existence of three oxyaryl carbon resonances at δ 164.63, 163.55 and 163.28, two arylmethine resonances at δ 96.06 and 92.17 and an acetyl substituted non-protonated aryl carbon at δ 106.78. These ^{13}C NMR spectral features, together with the appearance of a pair of doublets at δ 6.02 and 6.00 with *meta* splitting ($J = 2.2$ Hz) in the ^{1}H NMR spectrum, were in accordance with the existence of a phloroglucinol type of substitution pattern [5–9]. The ^{1}H–^{1}H COSY combined with the ^{13}C–^{1}H HETCOR experiments permitted the unambiguous assignment of all the ^{13}C resonances and of all the glycosidic protons. Consideration of $^{3}J_{HH}$ coupling constants obtained from a resolution-enhanced 1D ^{1}H NMR spectrum, together with a comparison of the NMR data with those for model sugars, identified the monosaccharide residue as β-D-glucopyranose [10–12].

Acid hydrolysis of **1** afforded an aglycone (**2**), the ^{1}H NMR spectrum of which showed one D_2O exchangeable signal at δ 13.94, suggesting the presence of a chelated hydroxyl group. The ^{1}H NMR data also displayed a pair of *meta*-coupled doublets ($J = 2.2$ Hz) at δ 6.03 and 5.94, a methoxyl (δ 3.90) and an acetyl methyl group (δ 2.55). The appearance of six aryl resonances in the ^{13}C NMR spectrum reflected an unsymmetrical substitution pattern of oxy substituents (2 × OH, 1 × OMe) on an acetophenone skeleton. A comparison of the observed ^{13}C NMR data with the reported literature values for related acetophenones [5, 7, 13, 14] led to its identification as 2,4-dihydroxy-6-methoxyacetophenone [14]. The fact that the acetyl methyl carbon resonance remains almost unaffected in **1** and **2** (**1**: δ 32.33; **2**: 32.77) also supported the existence of a free hydroxyl group at C-2 [8, 15].

In order to establish the glucose–aglycone linkage, the UV spectral data and ^{13}C NMR chemical shifts for

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1 and **2** were compared. The UV spectra of **2** showed two absorption bands at 286 and 220 nm. The absorption band at 286 nm showed a shift to 316 nm upon addition of sodium acetate, reflecting the presence of a free hydroxyl group at the C-4 position. The fact that the UV spectrum of **1** did not show any sodium acetate induced shift inferred the lack of a free 4-hydroxyl group in **1** and hence its involvement in the *O*-glycosidic linkage. An upfield shift of C-4 (3.53 ppm) and a downfield shift (0.82 ppm) of C-1 observed in the ^{13}C NMR spectrum of **2** as compared with **1** were also in accordance with the above proposed glycosylation site. Thus, the structure of **1** is 2-hydroxy-4-*O*-β-D-glucopyranosyloxy-6-methoxyacetophenone.

2,4,6-Trioxygenated acetophenones (derivatives of phloracetophenone) are not common in nature. 2,4,6-Trimethoxyacetophenone has been reported in *Lycoris sanguinea* [16] and 2-hydroxy-4,6-dimethoxyacetophenone 2-*O*-β-D-glucoside and 4-hydroxy-2,6-dimethoxyacetophenone 4-*O*-β-D-glucoside from *Pancratium biflorum* [13]. 2,4-Dihydroxy-6-methoxyacetophenone has been characterized from *Inula viscosa* [14] and *Phagnalon purpurescence* [17], but it is now reported for the first time as the 4-glucoside. Finally, the 2-glucoside and 2-rhamnoglucoside of 2,6-dihydroxy-4-methoxyacetophenone have been reported earlier from *Rhamnus libanoticus* [18] and *Lepisorusthum bergianus* [19], respectively.

EXPERIMENTAL

General. All mps were determined in open capillaries and uncorr. The instruments in the study were used under the experimental conditions reported previously [20]. CC was carried out on E. Merck silica gel (60–120 mesh). Visualization of TLC plates was done using 10% H_2SO_4 spray reagent followed by heating.

Plant material and isolation. Aerial parts of *A. annua* were collected from CIMAP farm, Lucknow. After grinding and defatting, samples were extracted with EtOH (×3). The combined EtOH extract was concd *in vacuo*, diluted with H_2O and extracted successively with $CHCl_3$, EtOAc and *n*-BuOH. The concd *n*-BuOH extract (150 g) was fractionated by CC over silica gel with $CHCl_3$–MeOH mixts of increasing polarity as eluents, with 400 frs (500 ml each) being collected. Frs 137–145, eluted with $CHCl_3$–15% MeOH, afforded a residue (150 mg) which upon repeated crystallization afforded **1** (125 mg), mp 160–62°; $[\alpha]_D$ −0.4; IR ν_{KBr}: 3465, 1640; UV λ_{MeOH}: 280, 220; $\lambda_{MeOH+NaOAc}$: 280, 220; ^1H NMR (D_2O, at 70°, 400 MHz): δ 6.02, 6.00 (1H each, *d*, *J* = 2.2 Hz, H-3, H-5), 4.95 (1H, *d*, *J* = 6.9 Hz, H-1′), 3.79, 3.61 (1H each, H_2-6′), 3.69 (3H, *s*, OMe), 3.45–3.52 (3H, *m*, H-2′, H-3′, H-5′), 3.33 (1H, *dd*, H-4′), 2.34 (3H, *s*, Ac); ^{13}C NMR (D_2O, at 70°, 100 MHz): δ 205.72 (CO), 164.63 (C-4), 163.55, 163.28 (C-2, C-6), 106.78 (C-1), 99.18 (C-1′), 92.12, 96.06 (C-3, C-5), 76.35 (C-3′), 75.54 (C-5′), 72.75 (C-2′), 69.44 (C-4′), 60.59 (C-6′), 55.61 (6-OMe), 32.33 ($CO\underline{CH_3}$); EIMS *m/z*: $[M]^+$ 344 (7), 182 (50), 165 (100), 164 (10), 152 (11), 102 (5), 87 (9), 73 (8), 69 (12).

Acid hydrolysis of **1** *with TFA.* Acidified **1** (100 mg) was dissolved in TFA (5 ml) and refluxed for 4 hr. The reaction mixt. on usual work up afforded a residue which on crystallization (EtOH–H_2O) yielded **2** (55 mg), mp 207–209°; UV λ_{MeOH}: 286, 220; $\lambda_{MeOH+NaOAc}$: 316, 216; ^1H NMR (Me_2CO-d_6, 300 MHz): δ 13.94 (1H, *s*, OH-2), 6.03, 5.94 (1H each, *d*, *J* = 2.2 Hz, H-3, H-5), 3.90 (3H, *s*, OMe), 2.55 (3H, *s*, COMe); ^{13}C NMR (Me_2CO-d_6, 75 Hz): δ 203.3 (CO), 161.1 (C-4), 165.8, 164.7 (C-2, C-6), 107.6 (C-1), 96.5, 91.7 (C-3, C-5), 56.0 (OMe), 32.77 ($CO\underline{CH_3}$) identified as 2,4-dihydroxy-6-methoxyacetophenone [14].

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