

ANNPHENONE, A PHENOLIC ACETOPHENONE FROM *ARTEMISIA ANNUA**

ANIL K. SINGH, VIBHA PATHAK and PAWAN K. AGRAWAL†

Central Institute of Medicinal and Aromatic Plants, Lucknow 226 015, India

(Received in revised form 29 April 1996)

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 ¹³C NMR/DEPT data for **1** displayed 15 carbon atoms with 15 directly attached protons. The ¹³C 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 (¹³C: δ 99.18; ¹H: 4.95, 1H, *d*, *J* = 6.9 Hz), four hydroxymethines (¹³C: 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 C₁₅H₂₂O₉ 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 ¹H 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 ¹³C signals at δ 55.61 (methyl), 205.72 (C=O) and 32.33 (methyl) supported the above observations. The ¹³C 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 ¹³C 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 ¹H NMR spectrum, were in accordance with the existence of a phloroglucinol type of substitution pattern [5–9]. The ¹H–¹H COSY combined with the ¹³C–¹H HETCOR experiments permitted the unambiguous assignment of all the ¹³C resonances and of all the glycosidic protons. Consideration of ³*J*_{HH} coupling constants obtained from a resolution-enhanced 1D ¹H 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 ¹H NMR spectrum of which showed one D₂O exchangeable signal at δ 13.94, suggesting the presence of a chelated hydroxyl group. The ¹H 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 ¹³C NMR spectrum reflected an unsymmetrical substitution pattern of oxy substituents (2 × OH, 1 × OMe) on an acetophenone skeleton. A comparison of the observed ¹³C 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 ¹³C NMR chemical shifts for

*CIMAP publication No. 95-54J. Part 46 in the series 'Studies on Medicinal Plants'. For part 45 see ref. [1].

†Author to whom correspondence should be addressed.

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-Trioxxygenated 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 *Pancreatium 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 *Lepisorus thum 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 ($\times 3$). 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°; $[\alpha]_{\text{D}} -0.4$; IR $\nu_{\text{KB}}:$ 3465, 1640; UV $\lambda_{\text{MeOH}}:$ 280, 220; $\lambda_{\text{MeOH}+\text{NaOAc}}:$ 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 (COCH_3); EIMS *m/z*: $[\text{M}]^+$ 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 ($\text{EtOH-H}_2\text{O}$) yielded **2** (55 mg), mp 207–209°; UV $\lambda_{\text{MeOH}}:$ 286, 220; $\lambda_{\text{MeOH}+\text{NaOAc}}:$ 316, 216; ^1H NMR ($\text{Me}_2\text{CO-}d_6$, 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 ($\text{Me}_2\text{CO-}d_6$, 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 (COCH_3) identified as 2,4-dihydroxy-6-methoxyacetophenone [14].

Acknowledgements—We thank Prof. Sushil Kumar, Director (CIMAP), and Dr R. P. Sharma (Head, Phytochemical Technology Division) for their continued encouragement.

REFERENCES

- Khan, R. A., Agrawal, P. K. and Kapil, R. S. (1996) *Phytochemistry* **42**, 243.
- Woerdenbag, H. J., Lugt, C. B. and Pras, N. (1990) *Pharm. Weekbl. Sci.* **12**, 169.
- Woerdenbag, H. J., Pras, N., van Uden, W., Wallaart, T. E., Beekman, A. C. and Lugt, C. B. (1994) *Pharm. Weekbl. Sci.* **16**, 169.
- Yang, S. L., Roberts, M. F., Neill, M. J. O., Bucar, F. and Phillipson, J. D. (1995) *Phytochemistry* **38**, 255.
- Pelter, A., Ward, R. S. and Bass, R. J. (1978) *J. Chem. Soc., Perkin Trans I* 366.
- Ternai, B. and Markham, K. R. (1976) *Tetrahedron* **32**, 565.
- Dhami, K. S. and Stothers, J. B. (1965) *Can. J. Chem.* **43**, 479.
- Agrawal, P. K., Agarwal, S. K. and Rastogi, R. P. (1980) *Phytochemistry* **19**, 893.
- Agrawal, P. K., and M. C. Bansal (1989) in *Carbon-13 NMR of Flavonoids* (Agrawal, P. K., ed.), p. 283. Elsevier, Amsterdam.
- Agrawal, P. K., Bush, C. A., Qureshi, N. and Takayama, K. (1994) *Adv. Biophys. Chem.* **4**, 179.
- Agrawal, P. K., Jain, D. C., Gupta, R. K. and Thakur, R. S. (1985) *Phytochemistry* **24**, 2479.
- Agrawal, P. K. (1992) *Phytochemistry* **31**, 2207.
- Ghosal, S., Mittal, P., Kumar, Y. and Singh, S. K. (1989) *Phytochemistry* **28**, 3193.
- Grande, M., Piera, F., Cuenca, A., Torres, P. and Bellido, I. S. (1985) *Planta Med.* **51**, 414.
- Jensen, S. R., Nielsen, B. J. and Norn, V. (1977) *Phytochemistry* **16**, 2036.
- Takagi, S. and Yamaki, M. (1974) *Yakugaku Zasshi* **94**, 617.
- Zedro, C., Bohlmann, F. and Anderberg, A. A.

- (1991) *Phytochemistry* **30**, 3009.
18. Satake, T., Coskum, M., Hori, K., Saiki, Y. and Tanker, M. (1991) *Phytochemistry* **30**, 4191.
19. Hikino, H., Konno, C. and Takemoto, T. (1969) *Yakugaku Zasshi* **89**, 72.
20. Agrawal, P. K., Singh, A. K., Bhakuni, R. S. and Jain, D. C. (1995) *Curr. Res. Med. Arom. Plants* **17**, 321.