Pergamon

PII: S0031-9422(97)00035-6

Phytochemistry, Vol. 45, No. 4, pp. 839–840, 1997

0031-9422/97 $17.00+0.00

METHOXYLATED AURONES FROM *CYPERUS CAPITATUS*

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(*Received in revised form* 3 *December* 1996)

Key Word Index—*Cyperus capitatus*; Cyperaceae; flavonoids; aurones; aureusidin; 6,3′-dihydroxy-4,4′-dimethoxy-5-methylaurone; 4,6,3′,4′-tetramethoxylaurone.

Abstract—Aureusidin, 6,3′-dihydroxy-4,4′-dimethoxy-5-methylaurone and 4,6,3′,4′-tetramethoxyaurone were isolated from *Cyperus capitatus*. The compounds were identified by spectral means. ©1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In flavonoids, methylation represents a late step and occurs sporadically in nature; so far, only two methylaurones have been described: 4′-hydroxy-7-methylaurone 6-rhamnoside, from *Pterocarpus marsupium* [1], and 6,3′,4′-trihydroxy-4-methoxy-5-methylaurone from *Cyperus capitatus* Vandelli [2]. In a further analysis of this same species of *Cyperus*, another methylaurone, 6,3′-dihydroxy-4,4′-dimethoxy-5-methylaurone has been found (**1**). Also, a highly methoxylated, 4,6,3′,4′-tetramethoxyaurone (**2**) was isolated; it has the characteristic that it isomerises in solution, something that has not been described before in aurones, as far as we are aware. Besides these two new compounds, the common aureusidin (**3**) was also detected. All the above compounds were isolated from the underground organs, which is not a common finding in the genus *Cyperus*.

RESULTS AND DISCUSSION

Compounds **1** and **2** were fluorescent yellow under UV light (366 nm) and remained unchanged when sprayed with NA (Naturstoffreagenz A) on TLC cellulose plates. R_f and R_t values remained unchanged after heating with 2 N HCl. Compound **1** exhibited a typical UV spectrum of an aurone with $\lambda_{\text{nm}}^{\text{MeOH}}$: MeOH 252, 268, 335, 395; +sodium methoxide: 284, 419 (slightly decreased in intensity, stable for 5 min); $+AlCl_3$: superimposable to that obtained with MeOH; +sodium acetate: 275, 400. Its ^1H NMR spectrum exhibited the pattern of a B ring of the catechol type with δ 7.48 (1H, *d*, $J = 1{,}5$ Hz, C-2′), 7.31 (1H, *dd*, $J = 8.5$ Hz and 1.5 Hz, C-6′), 7.02 (1H, *d*, $J = 8.4$ Hz, C-5′), and also signals at δ 6.61 and 6.59 (1H each, for C-7 and benzylic proton), δ 4.05 (*s*, 3H, A ring $-OCH_3$), δ 3.83 (*s*, 3H, B ring $-OCH_3$) and δ 1.98 (*s*, 3H, Ar-CH_3). Mass spectra, with value of 328 *m/z* for $[M]^+$ and showing some of the characteristic ions of flavonoid breakdown is compatible with above data. Values from ^{13}C NMR (Experimental) point to a structure with an A-ring identical to the one of the methylaurone already isolated from this same species, that is, a 4-OMe, 5-Me, 6-OH substitution, confirmed, namely, by the absence of a reaction with $AlCl_3$. The B-ring methoxyl must be located on C-4′ due to weak reaction of the compound with sodium methoxide. ^1H NMR [3] and ^{13}C NMR spectral analysis [4] lead to the same assumption.

Compound **2** is a less polar aurone. Its mass spectrum, with a molecular ion of 342 *m/z* points to a flavonoid with four methoxyl groups. Data from ^{13}C and ^1H NMR clearly confirm the presence of a methoxylated aurone with the common oxygenation pattern, that is, **2** is 4,6,3′,4′-tetramethoxyaurone. The most important characteristic of this compound is that in solution, and due to the effect of light, it exists as an equilibrium of two isomers, *Z* and *E*; the compound always gave two spots on TLC and two peaks on HPLC. From HPLC and ^1H NMR data, the equilibrium ratio is about 2:1. Although the two isomers were easily separated, it was not possible to isolate them completely due to isomerization. However, working in the dark and reducing to the minimum the time of contact with methanol, it was possible to obtain a mixture where the *E* form was predominant. A ^1H NMR spectrum was recorded with this mixture. On comparing this spectrum ($E > Z$) with the one obtained in the equilibrium form ($Z > E$), it was possible to determine the signals of each isomer. In general, values of the chemical shifts obtained are in good agreement with those registered by Brady *et al.* [5] with synthetic aurones, the most important difference being in C-2′ with δ 7.55 (Z) and 8.48 (E). The ^{13}C NMR spectrum was registered with the mixture in equilibrium, but the spectrum obtained showed signals for the more stable and hence more abundant (Z) form.

Compound **3** was yellow on TLC cellulose plates, turned orange when sprayed with NA and presented all the characteristic values of aureusidin. UV-Vis, ^{13}C and ^{1}H NMR spectra were recorded and data obtained was in agreement with the literature [3]. Aueusidin has already been isolated from aerial parts of other species of *Cyperus* [6].

EXPERIMENTAL

Plant material and extraction. As in Ref. [2]

Isolation and purification. Isolation was achieved by column chromatography with Sephadex LH-20 and RP C18 Lobar, eluting with MeOH. Final purification was completed by semiprep. HPLC using a Spherisorb ODS-2 column (25 × 0.7 cm, 5 μm) using water and methanol. Elution was performed at a flow rate of 2 ml min^{-1}, starting with 40% MeOH and reaching 70% MeOH.

General methods. TLC, UV-Vis and ^{13}C and ^{1}H NMR spectra were recorded as in ref. [2].

Compound **1**. R_f: BAW (0.81), 60% HOAc (0.64). ^{13}C NMR: δ 145.96* (C-2), 178.75 (C-3), 156.87 (C-4), 117.04 (C-5), 165.68† (C-6), 93.30 (C-7), 165.30† (C-8), 105.31 (C-9), 110.29 (C-10), 124.94 (C-1′), 111.41 (C-2′), 146.61* (C-3′), 149.34 (C-4′), 112.16 (C-5′), 123.95 (C-6′), 61.39 (C4-OMe), 55.57 (C4′-OMe), 8.20 (C5-Me) (*†assignments may be reversed).

EIMS m/z (rel.int.): $C_{18}H_{16}O_6$, (found 328.1290, calcd: 328.0942)$[M]^+$ (100), 327 $[M-1]^+$ (39), 311 $[M-HO]^+$ (31), 299 $[M-HCO]^+$ (31), 297 $[M-OMe]^+$ (43), 181 $[A1+H]^+$ (9), 151 $[B2]^+$ (10), 148 $[B1]^+$ (28), 137 $[B4]^+$ (28), notation according to ref. [7].

Compound **2**. R_f values: Z form: BAW (0.80), 60% HOAc (0.70); E form: BAW (0.81), 60% HOAc (0.75); ^{1}H NMR: (Z form): δ 6.34 (1H, d, J = 1.7 Hz, C-5), 6.72 (1H, d, J = 1.7 Hz, C-7), 6.69 (1H, s, benzylic), 7.55 (1H, d, J = 1.7 Hz, C-2′), 7.07 (1H, d, J = 7.9 Hz, C-5′), 7.54 (1H, dd, J = 1.7 and 8.0 Hz, C-6′), methoxyl values: 3.923, 3.885, 3.833, 3.822; (E form) δ 6.29 (1H, d, J = 1.7 Hz, C-5), 6.51 (1H, d, J = 1.7 Hz, C-7), 7.00 (1H, s, benzylic), 8.48 (1H, d, J = 1.8 Hz, C-2′), 7.01 (1H, d, J = 7.9 Hz, C-5′), 7.54 (1H, dd J = 1.7 and 8.0 Hz, C-6′), methoxyl values: 3.894, 3.880, 3.839, 3816; ^{13}C NMR: δ 146.15 (C-2), 178.90 (C-3), 168.04* (C-4), 89.89 (C-5), 168.74* (C-6), 94.38 (C-7), 158.82 (C-8) 104.21 (C-9), 110.29 (C-10), 124.94† (C-1′), 111.89 (C-2′), 148.75 (C-3′), 150.27 (C-4′), 113.99 (C-5′), 124.79† (C-6′), four methoxyls: 55.59, 56.12, 56.42, 56.54 (*†assignments may be reversed). EIMS m/z (rel. int.): $C_{19}H_{18}O_6$ (found 342.1377, calcd 342.1098) $[M]^+$ (100), 341 $[M-1]^+$ (29), 327 $[M-CH_3]^+$ (25), 313 $[M-HCO]^+$ (22), 311 $[M-OH_3]^+$ (62), 151 $[B4]^+$ (19), 137 $[B_2-CO$ or $A_2-CO]^+$ (8).

Acknowledgement—We thank the Department of Chemistry of University of Aveiro for MS analysis.

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