Phytochemistry, Vol. 28, No. 7, pp. 1996–1997, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00

6-*C*-METHYL- AND 6,8-DI-*C*-METHYL-3,7-DI-*O*-METHYLKAEMPFEROL FROM *ALLUAUDIA DUMOSA*

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(*Received in revised form* 8 *November* 1988)

Key Word Index—*Alluaudia dumosa*; Didiereaceae; 5,4′-dihydroxy-3,7-dimethoxy-6-*C*-methylflavone; 5,4′-dihydroxy-3,7-dimethoxy-6,8-di-*C*-methylflavone.

Abstract—Two 3-methoxyflavones isolated from bark of *Alluaudia dumosa* have been identified as 5,4′-dihydroxy-3,7-dimethoxy-6-*C*-methylflavone and 5,4′-dihydroxy-3,7-dimethoxy-6,8-di-*C*-methylflavone.

INTRODUCTION

The bark and spines of Didiereaceae have been shown previously to contain many flavonoid aglycones, comprising a number of *C*-methylated compounds [1–9]. From the lipophilic portion of the extract from *Alluaudia dumosa* Drake bark, we have now isolated two additional *C*-methylflavonols. They have been identified by their UV, MS and ^{1}H NMR spectra.

RESULTS AND DISCUSSION

The UV spectrum of compound **1** in methanol and the shapes and positions of bands I and II after addition of $AlCl_3$ and $AlCl_3$ + HCl suggested that this was a 3-methoxyflavone with a free hydroxyl at C-5, mono-substituted on the B-ring and substituted at C-6, while the UV spectra in the same reagents indicated that **2** possessed a supplementary substituent at C-8 [10]. Lack of free 7-hydroxyl in both compounds was suggested by the absence of shift of band II after addition of sodium acetate [11] and the absence of band III after addition of sodium hydroxide [12]. Furthermore the latter reagent revealed the presence of free hydroxyl at C-4′ in the two cases. Accordingly, the first compound appeared to be a 6,7-disubstituted derivative of 3-methoxykaempferol and the second a 6,7,8-trisubstituted derivative of the same aglycone.

The mass spectrum of **1** exhibited a molecular peak at *m/z* 328 (100%) in accord with a flavonol containing two hydroxyl and three methyl groups ($C_{18}H_{16}O_6$). As the ^{1}H NMR spectrum showed one singlet at δ 2.01 (3H) and two singlets respectively at δ 3.80 and 3.91, the compound is substituted by one aryl methyl group and two methoxy groups. In the mass spectrum, the presence of a peak at *m/z* 181 (8%) indicated that the A ring bears two methyl groups [13]. In accord with the previous UV data, one is located at C-6 and the second at C-7. This compound is therefore 5,4′-dihydroxy-3,7-dimethoxy-6-*C* methylflavone.

With a molecular peak at *m/z* 342 (100%), the second compound appeared to be a 8-*C*-methyl derivative of **1**. This structure was confirmed both by the mass spectrum with an ion fragment at 195 (A ring with three methyl groups) and by the ^{1}H NMR spectrum which exhibited two singlets at δ 2.11 (3H) and 2.31 (3H) and two singlets at 3.75 (3H) and 3.80 (3H). This compound is thus 5,4′-dihydroxy-3,7-dimethoxy-6,8-di-*C*-methylflavone.

These two compounds have apparently been reported as constituents of *Kalmia latifolia* by Wollenweber and Kohorst [14]. But the UV and MS data—corresponding to our compound **2**—published by these authors do not agree with those found from *Alluaudia* and, indeed, are open to criticism. First, in methanol, the band I of 3-methoxyflavones with free hydroxyls at C-5 and C-4′ cannot been located at 315 nm as indicated by these authors; the λ_{max} of this band is situated at 332–336 nm. Secondly, after addition of $AlCl_3$, neutral or acidic, these authors do not observe the shift of the band I ($AlCl_3$: 310 nm; $AlCl_3$ + HCl: 309 nm) as expected for flavones or 3-methoxyflavonols with a free hydroxyl at C-5 [11]. Furthermore, in MS, the molecular peak at *m/z* 342 is not the main peak (only 30%). All these spectral data do not agree with the fundamental spectral rules and need revision.

Some spectral data of 5,4′-dihydroxy-3,7-dimethoxy-6-*C*-methylflavone, published previously, have to be completed or corrected. The UV data, after addition of $AlCl_3$, are incomplete: 3-methoxyflavones without 3′,4′-*o*-dihydroxy system and with a free hydroxyl at C-5 and substituted at C-6 by a *C*-methyl group present a split of the band I, the band Ia appearing as an inflexion at *ca* 405 nm. Moreover, in the MS as previously, the main peak is the molecular peak.*

*Note added in proof: while this manuscript was in press, we had the opportunity to compare the products isolated from *Kalmia* [14] directly with those we isolated from *Alluaudia*, as reported here and we found them to be identical. Hence the structures published earlier [14] were correct, although the UV data, in particular those for 5,4′-diOH-3,7-diOMe-6-Me flavone are erroneous. Moreover, the MS data reported in ref. [14] have also to be corrected.

These two compounds, besides the 13 *C*-methylflavonols or 3-methoxyflavonols [1–7], two *C*-methylflavanones [8, 9] and five 6-methoxyflavonols or 3-methoxyflavonols [15, 16], previously identified from Didiereaceae, shown once more the biosynthetic originality of this family.

From a chemotaxonomic point of view it is interesting to note that, among the different species of Didiereaceae, only *A. dumosa* possesses the two parent flavonoids, e.g. 6-*C*-methyl and 6,8-di-*C*-methylkaempferol. Moreover the *O*-methylation at position 7, only found in *A. dumosa* and *A. humbertii*, is noteworthy.

EXPERIMENTAL

The plant material used in this study was collected in the South of Madagascar in 1987. Dry powdered bark material (800 g) was extracted with Et_2O. This extract was concd under red. pres. to dryness. The residue was dissolved in MeOH. Column chromatography of this crude extract on Polyamide SC6 first with C_6H_6 as eluent then with a gradient C_6H_6–MeCOEt–MeOH provided 50 fractions of 500 ml each. Fractions nos 6 to 8 containing exclusively C_6H_6 were chromatographed by prep. TLC over Polyamide DC6 using C_6H_6–MeCOEt–MeOH (10:1:1) and gave two bands; after elution, each band was chromatographed over Polamid with C_6H_6–$CHCl_3$–MeCOEt–MeOH (30:30:1:1). Final purification was achieved by chromatography on LH 20 Sephadex column.

Compound **1**. λ_{max}^{MeOH} nm: 270 (2.01), 336 (2.04); $+AlCl_3$: 272 (0.76), 305sh, 348 (0.72), 407 i; $+AlCl_3+HCl$: 273 (0.63), 305sh, 348 (0.62), 405 i; +NaOH: 270 (0.53), 300sh, 392 (0.83, stable); +NaOAc: 270 (0.87), 300sh, 390 (1.01); $+NaOAc+H_3BO_3$: 270 (0.79), 337 (0.73). ^{1}H NMR: DMSO, 300 MHz: δ (ppm/TMS) 8.00 (2H, *d*, J = 9 Hz, H-2′, 6′), 6.96 (2H, *d*, J = 9 Hz, H-3′, 5′), 6.84 (1H, *s*, H-8), 3.91 (3H, *s*, OMe), 3.80 (3H, *s*, OMe), 2.01 (3H, *s*, C-Me); SM: EIMS *m/z* (rel. int.): 328 (100%), 327 (73%), 309 (31%), 299 (15%), 297 (10%), 285 (33%), 181 (8%), 121 (38%).

Compound **2**. λ_{max}^{MeOH} nm: 275 (1.05), 331 (0.75), 365sh; $+AlCl_3$: 276 (0.79), 305 (0.60), 358 (0.78), 415 i; $+AlCl_3+HCl$: 274 (0.71), 304 (0.56), 349 (0.64), 411 (0.28); +NaOAc: 255sh, 270 (1.2), 394 (1.16); $+NaOAc+H_3BO_3$: 272 (1.3), 329 (0.9), 365sh; +NaOH: 253 (0.9), 274 (0.95), 396 (1.21) stable. ^{1}H NMR: 8.01 (2H, *d*, J = 8,8 Hz, H-2′, 6′), 6.98 (2H, *d*, J = 8,8 Hz, H-3′, 5′), 3.80 (3H, *s*, OMe), 3.75 (3H, *s*, OMe), 2.31 (3H, *s*, C-Me), 2,11 (3H, *s*, C-Me). SM: 342 (100%), 341 (54%), 327 (10%), 323 (19%), 313 (7%), 309 (6%), 299 (28%), 297 (10%), 195 (4%), 157 (12%), 121 (22%).

Acknowledgements—The authors are grateful to U.N.E.S.C.O. for financial support (Proj. MAG/84/013) and for a grant (R.R.).

REFERENCES

1. Rabesa, Z., Voirin, B., Favre-Bonvin, J. and Lebreton, P. (1978) *Phytochemistry* **17**, 1810.
2. Rabesa, Z. and Voirin, B. (1978) *Tetrahedron Letters* **39**, 3717.
3. Rabesa, Z. and Voirin, B. (1979) *Phytochemistry* **18**, 692.
4. Rabesa, Z. and Voirin, B. (1979) *Z. Pflanzenphysiol.* **91**, 183.
5. Rabesa, Z. and Voirin, B. (1979) *C. R. Acad. Sci. C* **289**, 167.
6. Rasamoelisendra, R., Voirin, B., Favre-Bonvin, J., Andriantiferana, M. and Rabesa, Z. (1985) *Die Pharmazie* **40**, 59.
7. Rabesa, Z. and Voirin, B. (1985) *C. R. Acad. Sci.* **301**, 351.
8. Rabesa, Z. and Voirin, B. (1983) *Phytochemistry* **22**, 2092.
9. Voirin, B., Rasamoelisendra, R., Favre-Bonvin, J., Andriantiferana, M. and Rabesa, Z. (1986) *Phytochemistry* **25**, 560.
10. Voirin, B. (1983) *Phytochemistry* **22**, 2107.
11. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
12. Bacon, J. D., Mabry, T. J. and Mears, J. A. (1976) *Rev. Latinoam. Quim.* **7**, 83.
13. Audier, H. (1966) *Bull. Soc. Chim. Fr.* **9**, 2892.
14. Wollenweber, E. and Kohorst, G. (1984) *Z. Naturforsch.* **39c**, 710.
15. Rabesa, Z. and Voirin, B. (1979) *Phytochemistry* **18**, 360.
16. Rabesa, Z. and Voirin, B. (1980) *Phytochemistry* **19**, 710.