Pergamon

PII: S0031-9422(97)00739-5

Phytochemistry, Vol. 47, No. 7, pp. 1387–1391, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

A NOVEL RETRODIHYDROCHALCONE FROM THE STEM BARK OF *UVARIA MOCOLI*

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(*Received* 19 *May* 1997)

Key Word Index—*Uvaria mocoli*; Annonaceae; chalcones; flavanones; dihydrochalcones; oxoaporphine alkaloids.

Abstract—Investigation of the ethyl acetate extract of the stem bark of *Uvaria mocoli* resulted in the isolation of the novel retrodihydrochalcone 2-hydroxy-4,5,6-trimethoxydihydrochalcone together with the known flavonoids 2′-hydroxy-4′,6′-dimethoxychalcone, 2′-hydroxy-4′,5′,6′-trimethoxychalcone, 5,7-dimethoxyflavanone and 5,7,8-trimethoxyflavanone, the oxoaporphines lysicamine, liriodenine and isomoschatoline, benzoic acid and a mixture of sitosterol and stigmasterol. All the compounds were identified by analysis of their spectral data.

INTRODUCTION

The genus *Uvaria* L. (Annonaceae) comprises some 150 species [1]. About 20 of these have so far been investigated and have yielded a wide range of secondary metabolites with interesting biological activities [2], including sesquiterpenes [3, 4], indole derivatives [5, 6], 1-benzyltetrahydroisoquinoline derivatives [1, 7], benzyl benzoate esters [8], cyclohexene epoxides [9, 10], flavonoids [11, 12] and acetogenins [13, 14]. We have examined the stem bark of *Uvaria mocoli* De Wild. & Th. Dur., a large woody climber which grows in the tropical forest zone of west Africa between Sierra Leone and Zaire [15], and in this paper report the isolation of five flavonoids, three oxoaporphines, benzoic acid and a mixture of sitosterol and stigmasterol. Among the flavonoids is the novel retrodihydrochalcone 2-hydroxy-4,5,6-trimethoxydihydrochalcone (**3**).

RESULTS AND DISCUSSION

The ethyl acetate extract of the stem bark of *U. mocoli*, through a series of chromatographic fractionations, yielded the flavonoids **1**–**5**, the oxoaporphines alkaloids **6**–**8**, benzoic acid and a mixture of sitosterol and stigmasterol. The ^{1}H NMR spectra of **1** and **2** showed the presence of an unsubstituted B-ring and the characteristic *trans*-propenone moiety and a 2′-hydroxylated ring A of a chalcone [16]. Compound **1** further showed signals for two aromatic methoxyl groups and two protons *meta*-coupled to each other, which identified it as 2′-hydroxy-4′,6′-dimethoxychalcone [17]. Compound **2** differed in showing a single aromatic proton and three methoxyl singlets. It was identified as 2′-hydroxy-4′,5′,6′-trimethoxychalcone [18] rather than 2′-hydroxy-3′,5′,6′-trimethoxychalcone, on the basis of the ^{13}C NMR chemical shift values for the methoxyl carbons (δ 62.1, 61.5, 56.3) which revealed that two of them were sterically hindered by being flanked by substituents in both *ortho* positions [20, 21].

The ^{1}H NMR spectra of **4** and **5** showed the typical AMX system of flavanones [16] and an unsubstituted ring B. Based on the spectral characteristics they were identified as 5,7-dimethoxyflavanone [19] and 5,7,8-trimethoxyflavanone [20], respectively. Evidence for the substitution pattern of **5** was again derived from the ^{13}C NMR spectrum which revealed that only one of the methoxyl groups was sterically hindered (δ 61.7, 56.5, 56.3), whereas for the 5,6,7-methoxylation pattern two would be hindered.

The novel compound **3** was isolated as a yellow-brown amorphous solid. The UV spectrum showed maxima at 228, 275 and 307 nm, suggesting an extended benzyl chromophore and the IR spectrum showed hydroxyl and carbonyl absorptions. The HREI mass spectrum gave a molecular ion at *m/z* 316.1186 which solved for $C_{18}H_{20}O_5$. The ^{1}H NMR spectrum (Table 1) indicated the presence of an unsubstituted aromatic ring, an aromatic proton singlet, a broad proton singlet for a hydroxyl group, three

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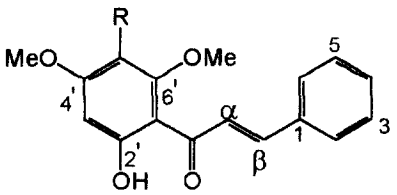
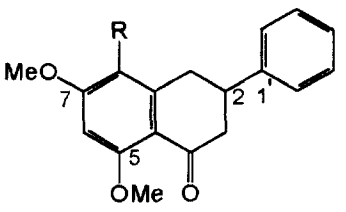
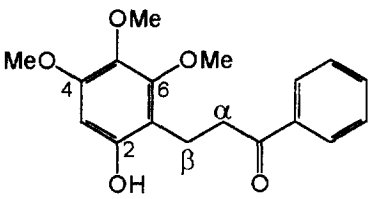


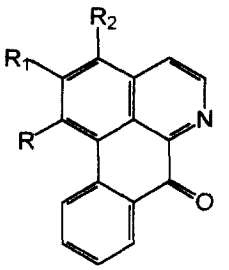
	R
1	H
2	OMe



	R
4	H
5	OMe



3



	R	R_1	R_2
6	OMe	OMe	H
7	-O-CH_2-O-		H
8	OMe	OMe	OH

Table 1. ^{1}H and ^{13}C NMR data ($CDCl_3$) and some significant ^2J and ^3J correlations in the HMBC spectrum of **3**

Position	δ_H*	δ_C†	$^2J_{H\text{-}C}$	$^3J_{H\text{-}C}$
1		113.5		
2		151.2		
3	6.35 *s*	97.7	δ 151.2 (C-2), δ 152.8 (C-4)	δ 113.5 (C-1), δ 136.0 (C-5)
4		152.8		
5		136.0		
6		152.3		
C=O		203.3		
C-α	3.43 *m*	17.5	δ 29.9 (C-β), δ 203.3 (C=O)	δ 113.5 (C-1), δ 136.1 (C-1′)
C-β	2.95 *m*	29.9	δ 17.5 (C-α), δ 113.5 (C-1)	δ 151.2 (C-2), δ 152.3 (C-6), δ 203. (C=O)
1′		136.3		
2′/6′	7.99 *m*	128.8		
3′/5′	7.44 *m*	128.6		
4′	7.59 *m*	134.0		
2-OH	8.45			
4-OMe	3.80	55.9		δ 152.8 (C-4)
5-OMe	3.78	61.0		δ 136.0 (C-5)
6-OMe	3.95	61.0		δ 152.3 (C-6)

* Solutions were referenced to $CHCl_3$ at δ 7.27 (400 MHz).
† Solutions were referenced to $CHCl_3$ at δ 77.23 (100 MHz).

methoxyls and two methylene multiplets. The ^{13}C NMR spectrum (Table 1) revealed six aromatic methines, five of which contributed to the unsubstituted phenyl ring, six quaternary aromatic carbons, a carbonyl at δ 203.3, two methylenes and three methoxyl signals. These data suggested a dihydrochalcone structure for **3**.

Examination of the unsubstituted aromatic moiety revealed that the two aromatic proton multiplets *ortho* to the ring junction (H-2′/H-6′) are relatively deshielded (δ 7.99) compared to those of compounds **1** (δ 7.61) and **2** (δ 7.66), suggesting the inductive effect of an adjacent C=O function and hence a benzoyl partial structure in **3**. This was confirmed by the ion at *m/z* 105 in the EI mass spectrum. Thus, **3** was a dihydrochalcone with substitution in ring B and not in ring A. In the ^{13}C NMR spectrum, two equivalent methoxyl carbons resonated at δ 61.0, with the other at δ 55.9, suggesting that the latter must be placed *ortho* to a proton while the former two, which are both sterically hindered, must be flanked by the ring junction and/or *ortho* oxygenated functions [20, 21]. This suggested that **3** was either 2-hydroxy-4,5,6-trimethoxydihydrochalcone or 4-hydroxy-2,5,6-trimethoxydihydrochalcone. The structure was established from 2D NMR experiments including HMBC and NOESY.

In the HMBC spectrum (Table 1) the position of the carbonyl function was confirmed by the ^2J correlation to H_2-α and the ^3J correlations to H_2-β and H-2′/6′ protons. The three methoxyl singlets showed ^3J correlations to the signals at δ_C 136.0 (C-5), δ_C 152.8 (C-4) and δ_C 152.3 (C-6), leaving the other oxygenated carbon at δ 151.2 (C-2) for the hydroxyl substitution. The aromatic proton singlet showed ^2J correlations to one methoxyl-bearing carbon (C-4) and the hydroxyl-bearing carbon (C-2), and ^3J correlations to the ring junction carbon (C-1) and the shielded methoxyl-bearing carbon (C-5). The H_2-β also showed ^2J correlation to the same ring junction carbon and ^3J correlations to the hydroxyl-bearing carbon (C-2) as well as a methoxyl-bearing carbon (C-6). This established unambiguously the position of the OH at C-2 rather than C-4. In the NOESY experiment, an nOe correlation was observed between the protons of the 4-OMe and H-3, and H_2-α and H-2′/6′. Thus, **3** was identified as 2-hydroxy-4,5,6-trimethoxydihydrochalcone.

2-Hydroxy-4,5,6-trimethoxydihydrochalcone (**3**) belongs to the retro-dihydrochalcone group of flavonoids because of the phloroglucinol-like oxygenation pattern of ring A [22]. It has been shown by feeding experiments that these retrochalcones are formed through carbonyl transposition of the corresponding normal chalcones, so that ring A is derived from cinnamoyl CoA and ring B, from the acetate-malonate pathway [22, 23]. Consequently, the co-isolation of **3** with **2** is significant, as **2** could serve as the normal chalcone intermediate which undergoes 1,3-carbonyl transposition to give **3**. This is the first report of a retrodihydrochalcone in the Annonaceae but the retrochalcone, tepanone (2-hydroxy-3,4,6-trimethoxychalcone) has been isolated from the root and stem bark of *Ellipeia cuneifolia* [24] and the stem bark of *U. pandensis* [25].

Alkaloids **6–8** were also identified by analysis of their spectral data and comparison with those published as the known oxoaporphines lysicamine [26], liriodenine [26] and isomoschatoline [27] respectively. This is, surprisingly, the first report of oxoaporphine alkaloids in *Uvaria*.

EXPERIMENTAL

General

Mps: uncorr; UV: MeOH; IR: $CHCl_3$; ^1H and ^{13}C NMR: recorded on Bruker AMX-400 instrument; MS: AEI-MS 902 double focusing instrument with direct probe insert at 70 eV; Si gel CC and VLC: Merck 60, 230–400 mesh; Petrol is petroleum ether (bp 60–80°).

Plant material

The plant materials used for this study were collected from Gyakye (Ashanti Region) in Ghana by the Herbarium Unit of the Forestry Department, Kumasi, Ghana, and identified by comparison with herbarium specimens.

Extraction and isolation of compounds

The stem bark powder (0.5 kg) of *U. mocoli* was Soxhlet extracted with EtOAc and concentrated under reduced pressure to give 10.2 g of dry extract. VLC fractionation of the EtOAc extract (10 g) over Si gel, eluting with toluene, toluene-$CHCl_3$ mixtures, $CHCl_3$ and finally $CHCl_3$–MeOH mixtures, yielded four frs: A (up to 5% $CHCl_3$ in toluene), B (up to 10% $CHCl_3$ in toluene), C (up to 20% $CHCl_3$ in toluene), D (up to 20% MeOH in $CHCl_3$). Fr A was CC over Si gel, eluting with petrol and increasing the polarity with EtOAc; frs 28-41 were bulked together and subjected to *prep*-TLC (toluene : EtOAc 9 : 1) to give **1** (118 mg). Fraction B was treated as in fr A and frs 21–31 after *prep*-TLC gave **2** (41 mg). Fraction C was CC over Si gel and eluted with petrol, petrol EtOAc mixtures and EtOAc; frs 16–20 after *prep*-TLC (petrol : EtOAc, 8 : 2) gave sitosterol/stigmasterol (12 mg); frs 23–31 on *prep*-TLC (petrol : EtOAc, 8 : 2) yielded **3** (32 mg); frs 58–80 were further CC over Si gel using petrol–EtOAc mixtures and frs 13–24 yielded **4** (99 mg). The rest of the frs collected were pooled and further fractionated on *prep*-TLC (petrol : EtOAc, 8 : 2) to give benzoic acid (3 mg), **4** (11 mg) and **5** (9 mg). Fr D was VLC over Si gel and eluted with 10% $CHCl_3$ in petrol and increasing the polarity till 20% MeOH in $CHCl_3$. Frs 7-8 (from $CHCl_3$: MeOH 95 : 5) was fractionated by *prep*-TLC (petrol : EtOAc, 8 : 2) to give **6** (35 mg)

and **7** (12 mg). Fr 10 (10% MeOH in $CHCl_3$) was CC over Si gel and **8** was collected as a blue band (4 mg)

Benzoic acid

Amorphous precipitate, identity confirmed by co-TLC with authentic sample.

Sitosterol/Stigmasterol

Needle crystals, identity confirmed by co-TLC with authentic samples isolated in our laboratory.

2′-Hydroxy-4′,6′-dimethoxychalcone (**1**)

Yellow amorphous solid, mp 92–94° (lit. 91.5–92°, [17]), $[M]^+$ 284.1041 ($C_{17}H_{16}O_4$ requires 284.1049), UV, IR, ^1H NMR, ^{13}C NMR, MS, in agreement with published data [17].

2′-Hydroxy-4′,5′,6′-trimethoxychalcone (**2**)

Yellow amorphous solid, mp 99–101° (lit. 102° [18]); $[M]^+$ 314.1144 ($C_{18}H_{18}O_5$ requires 314.1154), UV, IR, ^1H NMR, ^{13}C NMR, MS, in agreement with published data [18].

2-Hydroxy-4,5,6-trimethoxydihydrochalcone (**3**)

Brown-yellow amorphous compound; Found: $[M]^+$ 316.1186 ($C_{18}H_{20}O_5$ requires 316.1311), UV λ_{max} (MeOH) 228, 275, 307 (*sh*) nm; IR ($CHCl_3$, cm^{-1}) 3332 (OH) 1668 (C=O); EIMS *m/z* (rel. int., %) 316 (44) (M^+), 301 (75), 197 (44) 184 (35), 153 (545), 105 (100), 77 (95).

5,7-Dimethoxyflavanone (**4**)

White amorphous solid, mp 166–166° (lit. 169–172°, [19]), $[M]^+$ 284.1044 ($C_{17}H_{16}O_4$ requires 284.1049), UV, IR, ^1H NMR, ^{13}C NMR, MS, in agreement with published data [19].

5,7,8-Trimethoxyflavanone (**5**)

White amorphous solid, mp 151–152° (lit 154° [20]), $[M]^+$ 314.1144 ($C_{18}H_{18}O_5$ requires 314.1154), UV, IR, ^1H NMR, ^{13}C NMR, MS, in agreement with published data [20].

Lysicamine (**6**)

Yellow amorphous solid; $[M]^+$ 291.0892, ($C_{18}H_{13}O_3N$ requires 291.0895); UV, IR, ^1H NMR, MS, in agreement with published data [26].

Liriodenine (**7**)

Yellow amorphous solid; $[M]^+$ 275.0582, ($C_{17}H_9NO_3$ requires 275.0582), UV, IR, ^1H NMR, MS, in agreement with published data [26].

Isomoschatoline (**8**)

Dark red amorphous powder; $[M]^+$ 307.0803, ($C_{18}H_{13}NO_4$ requires 307.0845), UV, IR, ^1H NMR, MS, in agreement with published data [27].

Acknowledgements—T.C.F. thanks the Association of Commonwealth Universities for the award of a scholarship

REFERENCES

1. Leboeuf, M., Cavé, A., Bhaumik, P. K., Mukherjee, B. and Mukherjee, R., *Phytochemistry*, 1982, **21**, 2783.
2. Parmar, V. S., Tyagi, O. D., Malhotra, A., Singh, S. K., Bisht, K. S. and Jain, R., *Natural Product Reports*, 1994, 219.
3. Muhammad, I. and Waterman, P. G., *Journal of Natural Products*, 1988, **51**, 719.
4. Weenen, H., Nkunya, M. H. H., Mgani, Q. A., Psathmus, M. A., Waibel, R. and Achenbach, H., *Journal of Organic Chemistry*, 1991, **56**, 5865.
5. Achenbach, H. and Raffelsberger, B., *Tetrahedron Letters*, 1979, 2571.
6. Muhammad, I. and Waterman, P. G., *Journal of Natural Products*, 1985, **48**, 571.
7. Panichpol, K., Waigh, R. D. and Waterman, P. G., *Phytochemistry*, 1977, **16**, 621.
8. Kodpinid, M., Sadavongvivad, C., Thebtaranonth, C. and Thebtaranonth, Y., *Phytochemistry*, 1984, **23**, 199.
9. Jolad, S. D., Hoffmann, K. H., Cole, J. R., Tempesta, M. S. and Bates, R. B., *Phytochemistry*, 1984, **23**, 935.
10. Nkunya, M. H. H., Weenen, H., Bray, D. H., Mgani, Q. A. and Mwasunbi, L. B., *Planta Medica*, 1991, **57**, 341.
11. Hufford, C. D. and Oguntimein, B. O., *Journal of Natural Product*, 1982, **45**, 337.
12. Nkunya, M. H. H., Achenbach H., Renner, C., Waibel, R. and Weenen, H., *Phytochemistry*, 1990, **29**, 1261.
13. Jolad, S. D., Hoffmann, J. J., Schram, K. H., Cole, J. R., Tempesta, M. S., George, R. K. and Bates, R. B., *Journal of Organic Chemistry*, 1982, **47**, 3151.
14. Chen, Y. and Yu, D. Q., *Journal of Natural Products*, 1996, **59**, 507.
15. Le Thomas, A., *Fam.* 16, *Annonacees* in *Flore du Gabon*, ed. A. Aubreville. Museum National d'Histoire Naturelle, Paris, 1969, p. 43.
16. Mabry, J. T., Markham, K. R. and Thomas, M. B., *The Systematic Identification of Flavonoids*. Springler-Verlag, New York, 1970.

17. Itokawa, H., Morita, M. and Mihashi, S., *Phytochemistry*, 1981, **20**, 2503.
18. Bohlmann, F., Mahanta, P. K. and Zdero, C., *Phytochemistry*, 1978, **17**, 1935.
19. Bick, I. R. C., Brown, B. B. and Hills, W. E., *Australian Journal of Chemistry*, 1972, **25**, 449.
20. Panichpol, K. and Waterman, P. G., *Phytochemistry*, 1978, **17**, 1363.
21. Makriyannins, A. and Fesik, S., *Journal of the American Chemical Society*, 1982, **104**, 6462.
22. Saitoh, T. and Shibata, S., *Tetrahedron Letters*, 1975, 4461.
23. Ayabe, S. and Furaya, T., *Journal of Chemical Society Perkin Transactions*, 1982, **1**, 2725.
24. Colegate, S. M., Din, L. B., Ghisalberti, E. L. and Latiff, A., *Phytochemistry*, 1992, **31**, 2123.
25. Nkunya, M. H. H., Waibel, R. and Achenbach, H., *Phytochemistry*, 1993, **34**, 853.
26. Guinaudeau, H., Leboeuf, M. and Cave, A., *Journal of Natural Products*, 1975, **38**, 275
27. Atti, S. A. E., Ammar, H. A., Phoebe, C. H. Jr., Schiff, P. L. Jr. and Slatkin, D. J., *Journal of Natural Products*, 1981, **45**, 476.