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A FURANOID DITERPENE, 10 α-HYDROXYCOLUMBIN, FROM *TINOSPORA MALABARICA*

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Key Word Index—*Tinospora malabarica*; Menispermaceae; terpenoids: 10 α-hydroxy columbin.

Abstract—A new furanoid diterpene, 10 α-hydroxycolumbin, was isolated from the fresh stems of *Tinospora malabarica*. Its structure was established on the basis of spectral studies.

INTRODUCTION

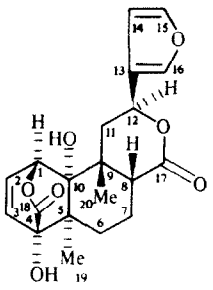
The plant family Menispermaceae consists of 65 genera and 350 species which are mainly tropical twining herbs, shrubs and trees. This family has long served as a rich source of alkaloids. *Tinospora malabarica* (Miers) belonging to this family is cultivated throughout Pakistan. The aqueous extract of the plant is used in the indigenous system of medicine for the treatment of intermittent fever [1, 2], liver and eye ailments and is reputed as a tissue builder and emetic [3]. A number of chemical constituents have been reported from this plant [4–6]. In the present communication we report the isolation and structure determination of a new furanoid diterpene, 10α-hydroxycolumbin, from the stems of *Tinospora malabarica*.

RESULTS AND DISCUSSION

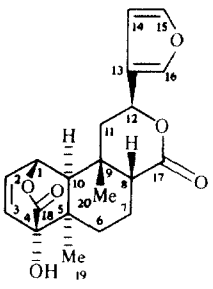
The compound **1** was isolated by column chromatography on a silica gel column and purified by preparative TLC to afford a white amorphous solid, 10α-hydroxycolumbin (**1**). Its UV spectrum showed absorption at λ_{max}^{MeOH} 215 nm indicating the presence of a furan ring [7–13]. The IR spectrum ($CHCl_3$) showed absorptions at 3460, 3490 cm^{-1} (OH), 1743, 1720 cm^{-1} (lactone), and 1505, 880 cm^{-1} (furan ring) [8–14]. The presence of a furan ring was also indicated by a positive Ehrlich colour test [15].

The substance was shown to have the formula $C_{20}H_{22}O_7$ on the basis of its molecular ion $[M]^+$ which appeared at *m/z* 374.133 (calc. 374.1326). The fragment ions at *m/z* 81 (corresponding to fission of C-11/C-12 bond to give ion **b**) and *m/z* 94 (fission of the C-9/C-11 bond to give ion *a*) clearly indicated that the furan ring occupies the usual position at C-12 as in other furanoid diterpenes. [5, 7, 13, 16, 17].

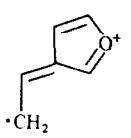
The ^1H NMR spectrum in acetone-d_6 showed a one-proton double doublet at δ5.66 ($J_{12,11\beta}$ = 11.5, $J_{12,11\alpha}$ = 5.0 Hz) which was assigned to the C-12 proton [9, 12, 13] and two double doublets at δ2.45 ($J_{11\beta,11\alpha}$ = 14.5, $J_{11\beta,12}$ = 11.5 Hz) and δ2.25 ($J_{11\alpha,11\beta}$ = 14.5, $J_{11\beta,12}$ = 5.0 Hz) each integrating for one proton, which were assigned to the C-11β and C-11α protons, respectively. Irradiation at δ5.66 (H-12) resulted in each of the double doublets at δ2.45 and δ2.25 collapsing to simple doublets. Similarly irradiation at δ2.45 and 2.25 (H-11β and H-11α) resulted in the collapse of the double doublet at



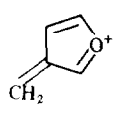
1



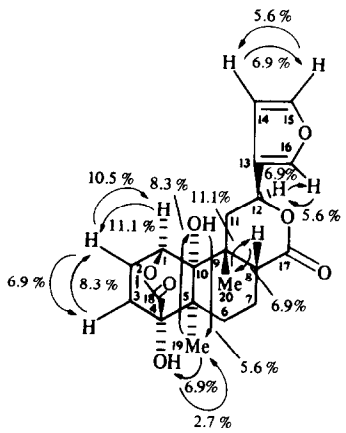
2



a *m/z* 94



b *m/z* 81



δ5.66 into a doublet. The two methyl groups at C-9 and C-5 were observed as 3H singlets at δ1.07 and 1.21 respectively [9, 10, 12, 13, 18]. The chemical shift of the methyl group at C-9 was observed at high field due to the influence of the furan ring on the same side as the C-9 methyl group. The position of the C-9 methyl group was also confirmed by NOE experiments (see Fig. 1). Three vicinal protons resonated as a double doublet at δ5.40 (H-1, $J_{1,2}=5.0$, $J_{1,3}=1.8$ Hz), a double doublet at δ6.52 (H-2, $J_{2,1}=5.0$, $J_{2,3}=7.8$ Hz) and a double doublet at δ6.25 (H-3, $J_{3,2}=7.8$, $J_{3,1}=1.8$ Hz), respectively. The C-8 proton was found to resonate as a double doublet at δ3.0 ($J_{8\beta,7\beta}=6.1$, $J_{8\beta,7\alpha}=9.5$ Hz) [14], while the C-6 and C-7 protons appeared together as overlapping multiplets at δ1.17.

Irradiation of the double doublet for the C-1 proton resulted in the collapse of the double doublet for the C-2 proton to a simple doublet, while irradiation of the double doublet for the C-2 proton resulted in the collapse of the double doublets for the C-1 and C-3 protons to simple doublets. Similarly irradiation of the double doublet for the C-3 proton resulted in the collapse of the double doublet for the C-2 proton to a simple doublet, establishing the connectivity between these protons. Irradiation of the C-8 proton resulted in a simplification of the multiplets for the C-7 protons whereas irradiation at the chemical shift of the C-7 protons caused a collapse of the double doublet of the C-8 proton into a doublet.

The aromatic protons of the furan ring system at C-14, C-15 and C-16 were found to resonate at δ6.63 (*dd*, $J_{14,15}=1.7$, $J_{14,16}=1.0$ Hz), 7.64 (*dd*, $J_{15,14}=1.7$, $J_{15,16}=1.7$ Hz), 7.71 (*dd*, $J_{16,15}=1.7$, $J_{16,14}=1.0$ Hz), respectively [7, 8, 12, 13]. The –OH protons appeared at δ6.00(*s*) and 6.50(*s*), which was confirmed by their disappearance on shaking with D_2O [7–11, 14, 17]. The second –OH was placed on the C-10 position as in other furanoid diterpenes, e.g. salviacoccin [14].

The ^{13}C NMR spectrum (acetone-d_6, 75.43 MHz) of 10α-hydroxycolumbin and its comparison with the ^{13}C NMR spectrum (DMSO-d_6, 25 MHz) of columbin is shown in Table 1. Gated Spin Echo experiments (GASPE) were carried out to determine the multiplicity of each carbon atom [19, 20].

The structure and stereochemical relationships were established by a series of NOE difference spectra. In the ^1H NMR spectrum of 10α-hydroxycolumbin, the signal at δ5.66 (H, *dd*, $J=5.0$, 11.5 Hz) was assignable to the proton attached to C-12 [12, 13]. Irradiation at the C-12 proton resulted in a 6.9% NOE of the C-16 proton, while irradiation at H-16 resulted in 5.6% NOE of the C-12 proton. Irradiation at C-20 methyl protons resulted in 11.1% NOE of the C-8 proton, while irradiation at H-8 resulted in 6.9% NOE of the C-20 methyl protons, establishing that the C-8 proton is β-oriented. Irradiation at the C-19 methyl protons resulted in 8.3% NOE of the C-10 hydroxyl and 6.9% NOE of the C-4 hydroxyl while irradiation at the C-10 hydroxyl resulted in 5.6% NOE of the C-19 methyl protons. Irradiation at the C-4 hydroxyl resulted in 2.7% NOE of the C-19 methyl protons, which established that the C-4 hydroxyl, C-19 methyl protons and C-10 hydroxyl are α-oriented in 10α-hydroxycolumbin (**1**). Similarly NOE at other points in the NMR spectrum (Fig. 1) served to establish the stereochemistry of 10α-hydroxycolumbin.

EXPERIMENTAL

CC: Merck silica gel 60 (7754), 70–230 mesh. TLC: silica gel GF-254 precoated plates (Merck).

Isolation of 10α-*hydroxycolumbin.* The fresh stems of *Tinospora malabarica* (120 kg) were crushed, extracted with EtOH (120 l) and evapd to a crude gum (300 g). The basic materials were removed by extraction with dil. HCl. The neutral fraction (30 g) was subjected to CC on silica gel (900 g). Increasing polarities of mixtures of petrol (bp 40–60) and Me_2CO were used as eluents. The fraction obtained on elution with petrol–Me_2CO (1:1) was evapd to dryness. This fraction was subjected to prep. TLC which resulted in the isolation of the new furanoid diterpene, 10α-hydroxycolumbin (**1**), $[\alpha]_D^{23}=+68.5°$

Table 1. ^{13}C NMR spectral values (δ) of 10α-hydroxycolumbin (acetone-d_6, 75.43 MHz) and (DMSO-d_6, 25 MHz) of columbin.

C	10α-Hydroxycolumbin	Columbin
1	74.65	73.0
2	131.16	130.0
3	137.41	135.0
4	81.50	80.2
5	37.20	36.7
6	27.52	25.6
7	17.42	16.9
8	44.85	43.2
9	35.63	34.5
10	71.85	45.6
11	40.78	39.9
12	71.03	70.0
13	125.50	124.9
14	109.70	108.9
15	140.85	140.0
16	143.75	143.3
17	173.50	174.2
18	172.50	173.1
19	27.52	27.0
20	24.86	23.6

(MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460, 3490 (OH); 1505, 880 (furan ring); 1743, 1720 (lactone); 1630 (double bond); UV λ_{max}^{MeOH} nm: 215 (furan ring). ^1H NMR (300 MHz, acetone-d_6 and acetone-d_6 plus D_2O): see Results and Discussion; Overhauser effect, irradiation at C-5 methyl group resulted in 11.1% NOE of C-10 hydroxyl while irradiation at C-10 hydroxyl resulted in 8.3% NOE of C-5 methyl (see Fig. 1) ^{13}C NMR (75.43 MHz, acetone-d_6): Table 1. High resolution MS: 374.133 $[M]^+$, (20%, calc. for $C_{20}H_{22}O_7$: 374.1326), 124.0875 (100%, calc. for $C_8H_{12}O$: 124.0888), 95.0492 (22%, calc. for C_6H_7O: 95.0496), 94.0417 (41%, calc. for C_6H_6O: 94.0418), 81.0345 (45%, calc. for C_6H_5O: 81.0340).

REFERENCES

1. Siddiqui, M. A. (1974) *Flora of West Pakistan* (Nasir, E. and Ali, S. I., eds) No. 74, p. 5. Ferozsons Press, Karachi.
2. Perry, L. M. (1980) *Medicinal Plants of East and Southeast Asia*, p. 268. MIT Press, Cambridge, Massachusetts.
3. Nadkarni, A. K. (1976) *Indian Materia Medica* Vol. 1, p. 1221. Popular Prakashan., Bombay.
4. Mehta, R., Arora, O. P. and Mehta, M. (1981) *Indian. J. Chem.* **20**, 834.
5. Atta-ur-Rahman, Ahmad, S. Malik, S. Shamma, M. and Freyer, A. J. (1988) (in press).
6. Bowen, I. H. and Motawe, H. M. (1985) *Planta Med.* 529.
7. Anthonsen, T., McCabe, P. H., McCrindle, R. and Murray, R. D. H. (1966) *Chem. Commun.* 740.
8. Eagle, G. A. and Rivett, D. E. A. (1973) *J. Chem. Soc. Perkin Trans. I* 1701.
9. Hori, T., Kiang, A. K., Nakanishi, K., Sasaki, S. and Woods, M. C. (1967) *Tetrahedron* **23**, 2649.
10. Ito, K. and Furukawa, H. (1969) *J. Chem. Soc. Chem. Comm.* 653.
11. Anthonsen, T., McCabe, P. H., McCrindle, R. and Murray, R. D. H. (1969) *Tetrahedron* **25**, 2233.
12. Yonemitsu, M., Fukuda, N., Kimura, T. and Komori, T. (1986) *Liebigs Ann. Chem.* 1327.
13. Fukuda, N., Yonemitsu, M. and Kimura, T. (1986) *Chem. Pharm. Bull.* **34**, 2868.
14. Savona, G., Bruno, M., Paternostro, M., Marco, J. L., Rodriguez, B. (1982) *Phytochemistry* **21**, 2563.
15. Reichstein, T. (1932) *Helv. Chim. Acta*, **15**, 1110.
16. Budzikiewicz, H., Djerassi, C. and Williams, D. H. (1964) *Structure Elucidation of Natural Products by Mass Spectrometry* Vol. II, 158. Holden-Day, San Francisco.
17. Ahmad, M., Khaleque, A. and Wahed Mian, M. A. (1978) *Indian J. Chem.* **16B**, 317.
18. Kusumoto, S., Okazake, T., Ohsuka, A. and Kotake, M. (1969) *Bull. Chem. Soc. Jpn* **42**, 812.
19. Benn, R. and Gunther, H. (1983) *Angew Chem. Int. Edn. Engl.* **22**, 350.
20. Wehrli, F. W. and Nishida, T. (1979) *Progress in Chemistry of Organic Natural Products* Vol. 36, (Herz, W., Grisebach, H. and Kirby, G. W. eds), p. 65. Springer, New York.

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A DITERPENE AND FLAVONOIDS OF *BACCHARIS FLABELLATA*

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Key Word Index—*Baccharis flabellata*; Compositae; *seco*-clerodane diterpenoid; *neo*-clerodane diterpenoid; oleanolic acid; flavonoids.

Abstract—From the aerial parts of *Baccharis flabellata*, two new clerodane type diterpenes were isolated together with oleanolic acid and four known flavonoids. The structures of the new compounds were elucidated by spectroscopic methods.

INTRODUCTION

Following our chemical study of the genus *Baccharis* (Compositae) [1–7], we have now investigated the constituents of *B. flabellata*. From the aerial parts of this plant we have isolated a new 5,10-*seco*-clerodane diterpenoid derivative, together with oleanolic acid and the four following flavonoids: 5,7,4′trihydroxy-6,3′-dimethoxyflavone (jaceosidin); 5,3′,4′-trihydroxy-6,7-dimethoxyflavone (cirsiliol); 5,7,3′,4′-tetrahydroxy-6-methoxyflavone (nepetin); 5,7,4′-trihydroxy-6-methoxyflavone (hispidulin). This paper describes the structural elucidation of the new compounds.