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MACROCYCLIC BISBIBENZYLS IN CULTURED CELLS OF THE LIVERWORT, *HETEROSCYPHUS PLANUS*

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Key Word Index—*Heteroscyphus planus*; Jungermanniales; suspension culture; macrocyclic bisbibenzyls; isoplagiochin; planusin A.

Abstract—Two bisbibenzyl compounds, isoplagiochin A and a novel bisbibenzyl compound, named planusin A, were isolated from cultured cells of the liverwort, *Heteroscyphus planus*. Their structures were determined on the basis of spectral analysis. © 1998 Elsevier Science Ltd. All rights reserved

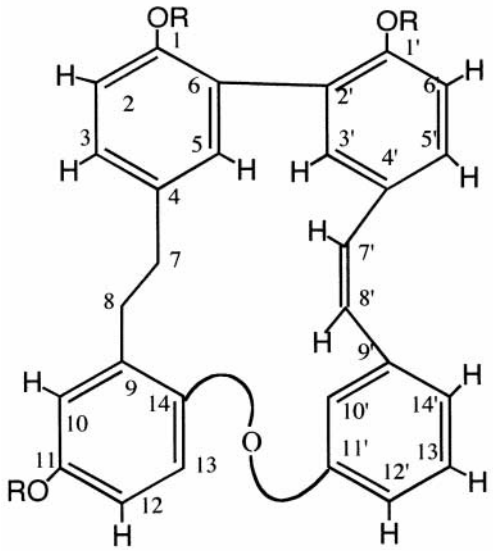
INTRODUCTION

We have previously reported the isolation of sesquiterpenes of the cadinane-type [1], alloaromandendrane-type and 2,3-secoalloaromandendrane-type [2], and diterpenes of the clerodane-type [3, 4] from cultured cells of *Heteroscyphus planus*. Hashimoto *et al*. [5] have recently reported diterpenes of the *epi*-neoverrucosane and *ent*-clerodane types, and sesquiterpenes of the *ent*-2,3-secoalloaromandendrane and cadinane types from *H. planus* harvested from the field. In a continuation of our work on secondary metabolites in cultured cells of the liverwort, *H. planus*, we have now isolated two macrocyclic bisbibenzyl compounds, isoplagiochin A (**1**) [6] and a novel compound (**3**), for the first time from cultured liverwort cells.

RESULTS AND DISCUSSION

The methanolic extracts of cultured cells and gametophytes were partitioned against pentane [1]. The pentane extract was further separated by sequential HPLC and liquid chromatography to give two macrocyclic bisbenzyls. One of them (**1**, HREI-MS *m*/*z*: 422.1542, calcd. for $C_{28}H_{22}O_4$, 422.1518) was identified as the known isoplagiochin A by comparison of its spectral data (IR, UV, ^{1}H and ^{13}C-NMR) with the published data for this compound [6]. The structure was supported by HMBC connectivities and the NOEs observed in NOE difference spectra.

A molecular formula of $C_{28}H_{22}O_4$ for bisbibenzyl **3** was determined by HREI-MS and from the ^{13}C NMR spectrum, edited by the DEPT pulse sequences, which afforded a total 28 resonance lines of two sp^3 methylenes, 15 sp^2 methines and 11 quaternary carbons including six carbons bearing oxygen atoms. In the ^{1}H NMR spectrum of **3**, two 1,2,4 trisubstituted rings [a set of ring A signals: $\delta 1_H$ 7.20 *dd* (J = 1.9 and 8.3 Hz, Table 1), 6.94 *d* (8.3) and 7.40 *d* (1.9), and of ring B signals: 7.08 *dd* (2.2 and 7.9), 6.80 *d* (7.9) and 6.54 *d* (2.2)], a 1,2,3-



1: R=H
2: R=Ac

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Table 1. ^{13}C (67.8 MHz)— and ^{1}H (270 MHz)—NMR spectral data for compound **3** in $CDCL_3$

C	δ ^{13}C	δ ^{1}H (*J* Hz)	C	δ ^{13}C	δ ^{1}H (*J* Hz)
1	138.5		1′	110.7	6.43 (*d*, 2.2)
2	130.8	7.20 (*dd*, 1.9 and 8.3)	2′	158.0	
3	117.0	6.94 (*d*, 8.3)	3′	116.2	7.49 (*m*)
4	125.3		4′	141.2	
5	133.2	7.40 (*d*, 1.9)	5′	124.0	6.80 (*d*, 8.3)
6	151.8		6′	126.0	7.14 (*t*, 8.3)
7	36.4	2.66 (*m*)	7′	128.5	6.63 (*d*, 12.4)
8	33.8	2.66 (*m*)	8′	130.4	6.66 (*d*, 12.4)
9	136.1		9′	136.2	
10	128.4	7.06 (*dd*, 2.2 and 7.9)	10′	127.8	
11	116.1	6.80 (*d*, 7.9)	11′	149.3	
12	150.0		12′	121.5	6.92 (*dd*, 1.5 and 7.9)
13	126.1		13′	130.1	7.15 (*d*, 7.9)
14	133.7	6.54 (*d*, 2.2)	14′	114.2	6.96 (*dd*, 1.5 and 7.9)

trisubstituted ring D [$\delta 1_H$: 6.92 *dd* (1.5 and 7.9), 7.19 *t* (7.9) and 6.96 *dd* (1.5 and 7.9)], and a 1,3-disubstituted ring C [$\delta 1_H$: 6.43 *d* (2.2), 7.49 *m*, 6.80 *d* (8.3) and 7.14 *t* (8.3)] whose presence were reinforced by ^{1}H-^{1}H homo-decoupling, ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and HMBC (Fig. 2) experiments. The arrangement of the substituents on the four benzene rings was established by NOE (Fig. 1) and HMBC experiments. Different NOEs were observed between H-3 and H-7, between H-5 and H-7, between H-8 and H-14, between H-8 and H-10, between H-8′ and H-14′, between H-3′ and H-7′, and between H-5′ and H-1′. Two quaternary carbon-bearing oxygen atoms, C10 and C11, could be automatically connected, while there were two possible ether linkages, between C-6 and C-2′ and between C-1 and C-2′. The ether linkage between C-6 and C-2′ is assigned to compound **3** as the sole ether linkage by taking account of steric distortion. Thus the structure of the unknown bisbibenzyl **3** was determined as indicated in Figs 1 and 2.

The production of macrocylic bisbibenzyls in aseptic gametophyte culture was reported previously by Adam and Becker [7].

EXPERIMENTAL

Isolation

Gametophytes grown on MSK-4 and AP-media (201 g and 180 g, fr. wt, respectively) and suspension cells (600 g, fr. wt.) were extracted with MeOH (×4) [1]. The combined MeOH soln was partitioned with *n*-pentane (×3). The resulting MeOH soln was evapd to dryness under reduced pres., and chromatographed on silica gel (1.5 kg). Elution of the column with *n*-hexane–EtOAc (7:3, 2400 ml) and then EtOAc (500 ml) gave frs containing the unknown bisbibenzyl **3** (frs 321–463) and isoplagio-

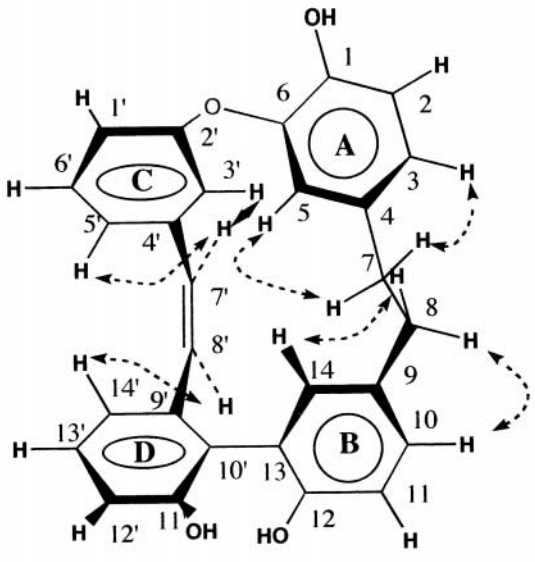

Fig. 1. Significant NOEs observed in compound **3**.

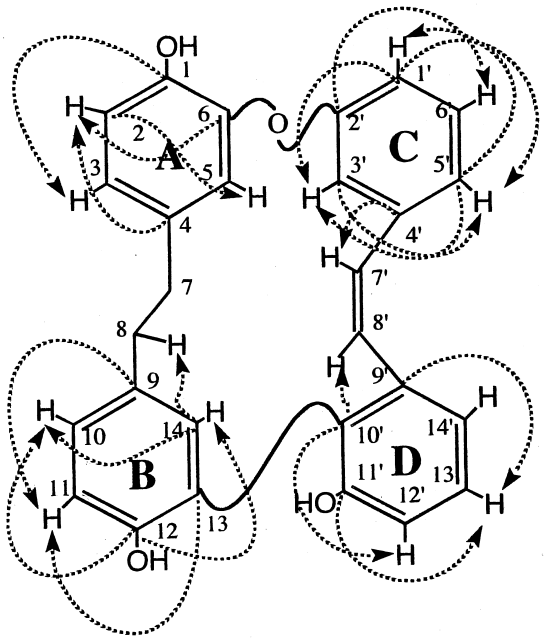

Fig. 2. HMBC connectivities observed in compound **3**.

chin A (**1**) (frs 464–519). Planusin A (**3** 1.7 mg) was isolated by successive HPLC of frs 321–463 on an ODS column (30 cm × 4.0 cm, i.d.) eluted with MeCN and a silica gel column (20 g) eluted with $CHCl_3$–EtOAc (10:1). Frs containing isoplagiochin were further sepd by HPLC on an ODS column (30 cm × 1.5 cm) eluted with MeCN, a silica gel column (15 g) eluted with $CHCl_3$–Me_2CO (9:1), and then by repeated HPLC on an ODS column (30 cm × 4 cm) eluted with MeOH–H_2O (7:3 and 5:2) to afford 2.9 mg of isoplagiochin A.

Isoplagiochin A

Optically inactive powder (*c* 0.193, MeOH); EIHR-MS *m/z* (rel int.); 422. 1542 $[M]^+$ (calc. for $C_{28}H_{22}O_4$: 422.1518) (100), 423 [M + 1] (31), 421 (11), 404 [M-H_2O] (21), 403 (11), 211 (13); UV λ_{max}^{EtOH} nm (log ϵ): 245 (4.22); 292 (4,11); IR υ_{max}^{KBr} cm^{-1}: 3400 (OH), 2900 (CH), 1550, 1385, 1240, 1024; ^1H NMR (270 MHz, $CDCl_3$): δ 2.68 (4H, *m*, H-7 and H-8), 6.36 (1H, *dd*, *J* = 7.9 Hz, H-12′), 6.57 (1H, *d*, *J* = 12.2, H-7′), 6.63 (1H, *d*, *J* = 12.2 Hz, H-8′), 6.67 (1H, *d*, *J* = 2.1 Hz, H-3′), 6.74 (1H, *m*, H-14′), 6.75 (1H, *dd*, *J* = 3.0 and 8.6, H-12), 6.79 (1H, *d*, *J* = 8.1 Hz, H-6′), 6.85 (1H, *d*, *J* = 3.0 Hz, H-10), 6.93 (1H, *d*, *J* = 8.3 Hz, H-2), 7.02 (1H, *d*, *J* = 8.6 Hz, H-13), 7.05 (1H, *dd*, *J* = 2.1 and 8.1 Hz, H-5′), 7.11 (1H, *t*, *J* = 7.9 Hz, H-13′), 7.19 (1H, *dd*, *J* = 2.2 and 8.3 Hz, H-3), 7.42 (1H, *m*, H-10′) and 7. 46 (1H, *d*, *J* = 2.2 Hz, H-5); ^{13}C NMR (67.5 Hz): δ 34.0 (C-8), 36.5 (C-7), 111.0 (C-12′), 114.0 (C-12), 116.0 (C-10′ and C-6′), 116.9 (C-2 and C-10), 122.7 (C-14′), 123.9 (C-13), 125.3 (C-6), 126.1 (C-2′), 128.4 (C-5′), 128.7 (C-3′), 129.7 (C-13′), 130.0 (C-4 and C-8′), 130.9 (C-3), 132.8 (C-5), 133.9 (C-7′), 136.1 (C-7′), 137.1 (C-9), 140.7 (C-9′), 145.0 (C-14), 150.0 (C-1′), and 152.3 (C-11) and 159.6 (C-11′). ^1H and ^{13}C-assignments were aided by DEPT, ^1H-^1H COSY, ^1H-^{13}C COSY, NOESY, different NOE amd HMBC experiments.

Isoplagiochin A triacetate (**2**)

Isoplagiochin A was acetylated in the usual manner. ^1H NMR (270 MHz, $CDCl_3$): δ 2.09, 2.15 and 2.33 (3 × 3H, 3 × *s*, 3× acetyl Me), 2.78 (2H, *m*, H-7), 2.81 (2H, *m*, H-8), 6.36 (1H, *dd*, *J* = 8.1 Hz and 2.5 Hz, H-12′), 6.59 (1H, *d*, *J* = 12.2 Hz, H-7′), 6.68 (1H, *d*, *J* = 12.2 Hz, H-8′), 6.67 (1H, *br d*, *J* = 7.4 Hz, H-14′), 6.89 (1H, *d*, *J* = 2.1 Hz, H-3′), 6.95 (1H, *d*, *J* = 8.1 Hz, H-6′), 7.02 (1H, *dd*, *J* = 2.7 and 8.3 Hz, H-12), 7.08–7.18 (5H, unresolved peaks, H-6′, H-10, H-2, H-5′ and H-13), 7.26 (unresolved peak, H-3), 7.49 (1H, *m*, H-10′) and 7.56 (1H, *d*, *J* = 2.1 Hz, H-5). ^1H assignments were based on ^1H-^1H COSY and ^1H-^1H homo-decoupling.

Planusin A (**3**)

Optically inactive powder (*c* 0.152, MeOH); EI-HR-MS *m/z* (rel int.); 422.1485 $[M]^+$ (calc. for $C_{28}H_{22}O_4$: 422.1518) (100), 423 [M + 1] (31), 421 (9), 404 [M–H_2O] (6); UV λ_{max}^{EtOH} (log ϵ): 233 (3.7), 279 (3.58), 297 (3.57), IR υ_{max}^{KBr}: 3450 (OH), 2900 (CH), 1600, 1550, 1498, 1250, 892.

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