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NEW BIBENZYLS FROM *RADULA COMPLANATA*

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Key Word Index—*Radula complanata*; Hepaticae; Jungermanniales; bibenzyls; isoprene unit; seven-membered heterocyclic ring.

Abstract—Two new bibenzyls have been isolated from *Radula complanata* and their structures have been elucidated by chemical and spectral evidence. In addition to the above two bibenzyls and four known bibenzyls, two new acidic bibenzyls have been isolated as their methyl esters.

INTRODUCTION

It is known that the European liverwort, *Radula complanata*, causes allergenic contact dermatitis [1], and it elaborates mono- and sesquiterpenes and 3-methoxybibenzyl [2]. Recently, we have found that the Japanese *Radula variabilis* contains various new bibenzyl derivatives, instead of the sesquiterpene lactones causing allergy [3, 4]. As part of our systematic investigation of the biologically active substances of bryophytes, we have reinvestigated the chemical constituents of *Radula complanata*. The present paper reports on the isolation and the elucidation of the structures of the new bibenzyls, together with the previously known bibenzyls with a unique seven-membered heterocyclic ring.

RESULTS AND DISCUSSION

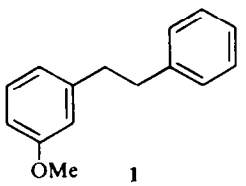
Air-dried *R. complanata* was extracted with MeOH. Combination of column and PLC on Si gel gave the bibenzyls (**1**, **2**, **3**, **7**, **8** and **9**), respectively. The two acidic bibenzyls (**11** and **13**) have been isolated as their methyl esters.

Bibenzyl (**2**)

The first bibenzyl, $C_{19}H_{22}O_2$ (M^+ 282), indicated the presence of a OH group (3400 cm^{-1}) and an aromatic group (1620, 1588 cm^{-1}; λ_{max} 211, 280 nm). Methylation of **2** gave the dimethyl ether (**4**), $C_{21}H_{26}O_2$ (M^+ 310), δ 3.87 ppm (*s*, 6H), indicating the presence of two OH groups in **2**. The presence of an unsubstituted benzyl group was indicated by the singlet signal at 7.32 ppm (5H) and by the base peak at *m/e* 91 ($C_7H_7^+$). The NMR spectrum also showed the presence of a phenolic OH group (2H) which disappeared on the addition of D_2O, four equivalent protons (2.88 ppm, *s*) characteristic of the bibenzylic methylenes and two equivalent aromatic protons at 6.35 ppm. The presence of a γ,γ-dimethylallyl group attached to the benzene ring was confirmed by the broad singlet signals at 1.78 (3H) and 1.87 ppm (3H), a broad triplet at 5.37 ppm (1H) and a broad doublet at 3.45 ppm (J = 6 Hz). All assignments were confirmed by a double resonance experiment. The presence of the γ,γ-dimethylallyl group was further supported by the prominent peak at *m/e* 227 ($M^+ - 55$). Treatment of **2** with HCl in HOAc afforded 2,2-dimethylchromane derivative (**5**), $C_{19}H_{22}O_2$ (M^+ 282), followed by methylation to give a monomethyl ether (**6**), $C_{21}H_{24}O_2$ (M^+ 296), indicating a OH group *ortho* to the isoprene chain. The equivalent chemical shift of the two aromatic protons showed the symmetrical substitution of the benzene ring and the γ,γ-dimethylallyl group and a phenethyl group being in *para* position and two OH groups being in *ortho* position with respect to the isoprene chain. The NOE experiment of the dimethyl ether (**4**) showed an increase in the intensity of the signal of the aromatic protons. On the basis of the above evidence, coupled with biogenetic considerations [5], the structure of the first new bibenzyl was established as 3,5-dihydroxy-4-(3-methyl-2-butenyl)-bibenzyl (**2**).

Bibenzyl (**3**)

The second bibenzyl, $C_{20}H_{24}O_2$, [M^+ 296], exhibited the presence of a OH group (3450 cm^{-1}) and one MeO group (δ 3.80 ppm). The signal pattern and chemical shifts in the NMR spectrum of **3** were strikingly similar to those of **2**, suggesting that **3** possessed the same skeleton as that of **2**, one OH group being replaced by



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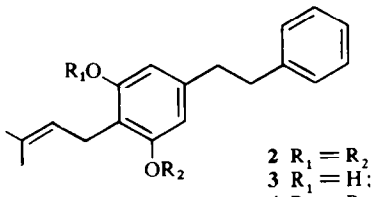

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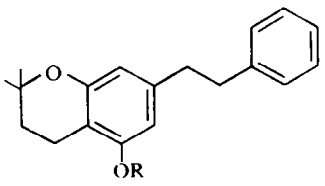
3 $R_1 = H$; $R_2 = Me$
4 $R_1 = R_2 = Me$



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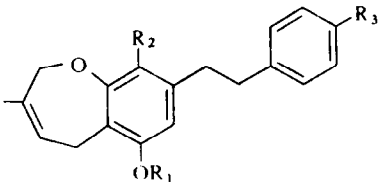
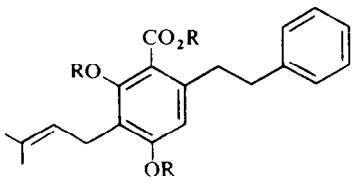


7 $R_1 = R_2 = R_3 = H$
8 $R_1 = R_2 = H$; $R_3 = OH$
9 $R_1 = R_3 = H$; $R_2 = COOH$
10 $R_1 = R_3 = H$; $R_2 = COOMe$



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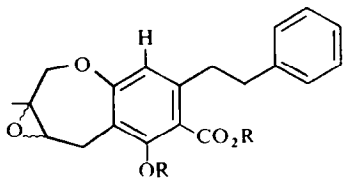

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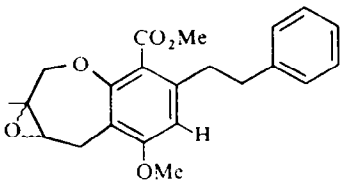

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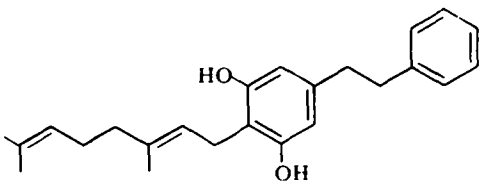
one OMe group. This was proved as follows. Methylation of **3** gave a dimethyl ether whose spectral and chromatographic behaviour were completely identical to those of the bibenzyl **4**. Furthermore, cyclization of **3** with HCl gave 2,2-dimethylchromane derivative whose spectral data were in accordance with **6**. Thus, the second bibenzyl is represented by **3**.

Bibenzyls (**7**), (**8**) and (**9**)

The four bibenzyls, after purification by column and PLC on Si gel were isolated pure. Their chemical and spectral properties were identical to those of the authentic bibenzyls (**7**), (**8**) and (**9**) isolated earlier from *R. variabilis* [3, 4].

Bibenzyls (**11**) and (**13**)

The two acidic bibenzyls (**11**) and (**13**) could not be isolated in the original state, because of difficulty in their separation. The mixture containing acidic bibenzyls indicated the presence of a carboxylic group (3500–2500; 1650 cm^{-1}) and an aromatic ring (1620, 1580 cm^{-1}). The NMR spectrum showed the absence of methoxyl groups and the presence of an unsubstituted benzene ring (7.25 ppm), dimethylallyl group (1.77, 1.85 ppm), aromatic proton (6.3 ppm) and tertiary methyl group (1.27 ppm). Methylation of this mixture with $(Me)_2SO_4$ followed by purification on TLC afforded the two pure methyl esters (**12**) and (**14**). The methyl ester (**12**), $C_{23}H_{28}O_4$, [M^+ 368), exhibited the IR band at 1730 cm^{-1}, due to methyl ester. The NMR spectrum contained a dimethylallyl group [1.68, 1.73 ppm, (each *bs*), 3H, 5.20 ppm (*m*), 1H, and 3.31 ppm (*bd*, $J = 7$), 2H] attached to a benzene ring, two OMe groups (3.78 and 3.95 ppm) and a carbomethoxyl group (3.78 ppm), one aromatic proton (6.43 ppm, *s*), four equivalent protons (2.90 ppm) characteristic of the bibenzylic methylenes and an unsubstituted benzene ring (7.28 ppm). The signal pattern and chemical shifts of the NMR spectrum of **12** were quite similar to those of the bibenzyl **4**, except the presence of the signal of one carbomethoxyl group, indicating that the bibenzyl **12** possessed the same skeleton as that of **4**, and one carbomethoxyl group was located on the benzene ring, instead of one aromatic proton. The above spectral data coupled with the similar cracking pattern of the mass spectrum to that of **4** and co-occurrence of the bibenzyls **2** and **3**, the methylated bibenzyl was established to be **12**, hence the acidic bibenzyl was deduced to be **11**. The methyl ester (**14**), $C_{22}H_{24}O_5$ (M^+ 368), indicated the IR band 1734 cm^{-1} due to COOMe group. The presence of the bibenzylic methylenes was confirmed by the singlet at 2.81 ppm (4H). The strong peak at *m/e* 91 (59%) and the signal at 7.20 ppm (5H) showed the presence of an unsubstituted benzyl group. The NMR spectrum also contained a OMe group (3.90 ppm) and a carbomethoxyl group (3.85 ppm), an aromatic proton (6.40 ppm, *s*), two nonequivalent protons of methylene group [4.16 ppm (*d*, $J = 10.5$) and 3.63 ppm (*d*, $J = 10.5$)] bearing ether oxygen, one tertiary methyl group (1.25 ppm, *s*) on carbon carrying an oxygen function, sp^3 proton (2.00 ppm, *m*) on carbon carrying oxygen function and a methylene (3.85 ppm, *m*) located between aromatic ring and sp^3 carbon. All assignments were confirmed by a double resonance experiment. The above results, together with the molecular formula indicated that in **14** one of the benzene rings was substituted by a carbomethoxyl group, a methoxyl group and isoprene unit which was cyclized by a phenolic OH group. The absence of a OH group in IR spectrum suggested that one of the five oxygen atoms was an epoxide function. The spectral data of **14** was very similar to those of the epoxide (**15**) derived from the bibenzyl (**10**), however, the ester **14** was more polar than **15**. The IR spectrum of the mixture of **11** and **13** contained the hydrogen bonded carboxylic group (1650 cm^{-1}) indicating the carboxylic group and phenolic OH group being placed in an *ortho* position in the original state. The above results, coupled with the co-occurrence of the bibenzyls (**7**), (**8**), (**9**) and (**11**), led us to **14** for the methylated bibenzyl and **13** for the original acidic bibenzyl



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The bibenzyl (**1**) is the major aromatic component of *R. complanata*. In *Radula variabilis*, the bibenzyl (**16**) is the major one. The compounds (**7**), (**8**) and (**9**) are common to both *Radula* species.

EXPERIMENTAL

UV, IR, NMR, MS, ORD curves and optical rotation were measured as reported in the preceding paper [4].

Extraction and isolation of bibenzyls. Radula complanata (L.) Dum. collected in Madirac (France) in November 1977, was washed with H_2O. After being air-dried for 2 days, the ground material (28 g) was extracted with MeOH for 2 weeks and the green viscous extract was directly chromatographed on Si gel using *n*-hexane and EtOAc gradient. The first fraction (n-hexane 100%) contained the mixture of *n*-paraffins and sesquiterpene hydrocarbons (56 mg) not identified. The second fraction (*n*-hexane–EtOAc, 19:1) gave the brownish oil (477 mg) which was rechromatographed on Si gel resulting in the isolation of the bibenzyl (**1**) (350 mg) and unsaturated fatty acid methyl ester (85 mg). The third fraction (9:1) gave the bibenzyl (**3**) (50 mg) and the bibenzyl (**7**) (139 mg). The bibenzyl (**3**): $C_{20}H_{24}O_2$, λ_{max} 211 nm (ε, 6046), 230 sh (3038), 270 (422); ν_{max} 3450 (OH), 1615, 1600, 1515 (Ar group), 1165, 1095, 700 cm^{-1} (unsubstituted C_6H_6 ring); δ 7.28 (*s*, 5H), 6.38 (*bs*, 1H), 6.32 (*bs*, 1H), 5.08 (*bs*, Ph—OH), 5.20 (*m*, 1H), 3.80 (*s*, OMe), 3.40 (*bd*, *J* = 7 Hz, 2H, Ph—C<u>H</u>$_2$—CH=), 2.90 (*s*, Ph—CH_2—CH_2—Ph), 1.83 (*bs*, 3H), 1.75 ppm (*bs*, 3H); *m/e* (%) 296 (M^+, 100), 281 (M − 15, 21), 241 (M − 55, 78), 205 (M − 91, 65), 91 ($C_7H_7^+$, 69). The bibenzyl (**7**): identical to the authentic sample. The fourth fraction (17:3) contained the bibenzyl (**7**) (20 mg) and the bibenzyl (**2**) (101 mg). The bibenzyl (**2**): $C_{19}H_{22}O_2$, λ_{max} 211 nm (ε, 13 442), 280 (7573); ν_{max} 3400, 1620, 1588, 1490, 1040, 750, 698 cm^{-1}; NMR (see text); *m/e* (%) 282 (M^+, 21), 227 (M − 55, 27), 191 (M − 91, 25), 91 (100). The viscous oil eluted by (3:1) was purified by PLC to give the bibenzyl (**8**) (237 mg), identical to the authentic sample. The fraction (3:2) contained the acidic bibenzyls (97 mg) which was methylated with $(Me)_2SO_4$ in the presence of K_2CO_3, followed by PLC (C_6H_6–EtOAc 4:1) to give the methyl ester (**12**) (58 mg) and **14** (18 mg). The bibenzyl (**12**): $C_{23}H_{28}O_4$, λ_{max} 212 nm (ε, 6781), 260 (1051); ν_{max} 1730, 1602, 1575, 1155, 1110, 975, 915, 845, 752, 700 cm^{-1}; *m/e* (%) 368 (M^+ 39), 353 (M^+ − 15, 9), 337 (M − 31, 29), 336 (M − 32, 43), 322 (M − 15 − 31, 23), 321 (M − 15 − 31, 100), 277 (M − 91, 56), 129 (22), 91 (79). The bibenzyl (**14**): $C_{22}H_{24}O_5$, $[\alpha]_D \pm 0°$; $[\phi]_{300-600\,nm}$ no curve; λ_{max} 216 nm (3827); ν_{max} 1734, 1618, 1575, 1500, 1310, 1270, 1157, 1100, 1065, 1035, 870, 750, 700 cm^{-1}; $\delta(C_6D_6)$ 7.10 (*s*, 5H), 6.60 (*s*, 1H), 3.80 (*s*, OMe), 3.60 (*s*, COOMe), 3.5 (overlapped 2H), 3.85 (*d*, *J* = 11, 1H), 3.33 (*d*, *J* = 11, 1H), 2.93 (*s*, 4H), 1.8 (*m*, 1H), 0.80 (*s*, 3H); *m/e* (%) 368 (M^+, 5), 352 (M − 16, 73), 337 (M − 16 − 15, 51), 321 (M − 16 − 15, 22), 261 (M − 16 − 91, 100), 105 (24), 91 (59).

The fraction (3:7–1:9) gave the pure acidic bibenzyl (**9**) (62 mg) identical to the authentic sample.

Methylation of **2**. The bibenzyl (**2**) (40 mg) in Me_2CO was methylated with $(Me)_2SO_4$ (0.1 ml) in the presence of K_2CO_3 (1 g) for 7 hr. Work up gave the dimethyl ether (**4**) (33 mg). $C_{21}H_{26}O_2$, λ_{max} 207 nm (ε, 4230), 239 (692); ν_{max} 1605, 1585, 1495, 1420, 1230, 1168, 1118, 746, 700 cm^{-1}; δ 7.38 (*s*, 5H), 6.48 (*bs*, 2H), 5.27 (*bt*, *J* = 7, 1H), 3.87 (*s*, 6H), 3.38 (*bd*, *J* = 7, 2H), 2.97 (*s*, 4H), 1.80 (*bs*, 3H), 1.70 (*bs*, 3H); *m/e* (%) 310 (M^+, 100), 295 (M − 15, 74), 279 (M − 31, 4), 255 (M − 55, 17), 219 (M − 91, 97), 105 (27), 91 (46).

Cyclization of **2**. The bibenzyl (**2**) (43 mg) in HOAc (2 ml) and conc. HCl (0.05 ml) was refluxed at 120° for 1 hr. The product after PLC (C_6H_6–EtOAc 4:1) was the chromane derivative (**5**) (18 mg) $C_{19}H_{22}O_2$, λ_{max} 203 nm (ε, 18 500), 233 sh (2820); ν_{max} 3400 (OH), 1627, 1587, 1495, 1158, 1122, 1058, 700 cm^{-1}; δ 7.28 (*s*, 5H), 6.35 (*bs*, 1H), 6.20 (*bs*, Ph—OH), 2.87 (*t*, *J* = 6, 2H), 1.80 (*t*, *J* = 6, 2H), 1.35 ppm (*s*, 6H, $(Me)_2C$—O—); *m/e* (%) 282 (M^+, 28), 227 (M − 55, 64), 191 (M − 91, 33), 174 (M − 91 − 17, 16), 105 (17), 91 (100).

Cyclization of **3**. The bibenzyl (**3**) (20 mg) was treated with HCl as indicated above to give a chromane derivative (**6**) (11 mg), $C_{20}H_{24}O_2$, λ_{max} 208 nm (6956), 232 sh (1726); ν_{max} 1620, 1585, 1495, 1385, 1370, 1230, 1165, 1110, 700 cm^{-1}; δ 7.12 (*s*, 5H), 6.26 (*bs*, 1H), 6.06 (*bs*, 1H), 3,76 (*s*, OMe), 2.86 (*s*, 4H), 2.56 (*t*, *J* = 6, 2H), 1.66 (*t*, *J* = 6, 2H), 1.30 (*s*, 6H) *m/e* (%) 296 (M^+ − 50), 241 (M − 55, 100), 205 (M − 91, 41), 91 (41).

Methylation of **5**. The chromane (**5**) (10 mg) was methylated with $(Me)_2SO_4$ to give the methyl ether whose spectral data and chromatographic behaviour was identical to those of **6**.

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