Pergamon

0031-9422(95)00025-9

Phytochemistry, Vol. 39, No. 3, pp. 613–616, 1995
Copyright © 1995 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0031-9422/95 $9.50 + 0.00

A SESQUITERPENE LACTONE, PHENYL AND BIPHENYL COMPOUNDS FROM *ANTRODIA CINNAMOMEA*

HUNG-CHEN CHIANG,* DE-PENG WU, I-WHA CHERNG and CHUEN-HER UENG

Institute of Chemistry, National Taiwan Normal University, Taipei 117, Taiwan, Republic of China

(*Received* 24 *October* 1994)

Key Word Index—*Antrodia cinnamomea*; sesquiterpene; antrocin; *Cinnamomum kanehirai.*

Abstract—Three novel compounds, a sesquiterpene, phenyl and biphenyl derivatives, have been isolated from the crude methanol extract of the fungus *Antrodia cinnamomea*, a new genus of *Antrodia* species. Their structures were all determined by spectroscopic data and confirmed by X-ray analysis.

INTRODUCTION

Antrodia cinnamomea Chang & Chou, sp. nov. growing rarely on the inner cavity wall of *Cinnamomum kanehirai* Hay. was identified as a new species of *Antrodia* in 1995 [1]. It was early identified as a new *Ganoderma* species, *Ganoderma camphoratum* in 1990 [2] because there were several similar characteristics. *A. cinnamomea* is well known in Taiwan under the name "niu chang ku" or "Jang-Jy" and is also popular and very expensive as medicinal material. It is used traditionally as an antidote, anticancer and antichromic material, but no biological activity tests have been reported. We report now the isolation and identification of three novel compounds, a sesquiterpene lactone (**1**) (antrocin), and the phenyl and biphenyl derivatives **2a** (4,7-dimethoxy-5-methyl-1,3-benzodioxole) and **3** (2,2′,5,5′-tetramethoxy-3,4,3′4′-bi-methylenedioxy-6,6′-dimethylbiphenyl), respectively.

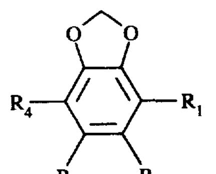
RESULTS AND DISCUSSION

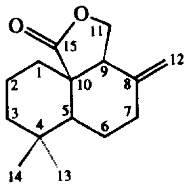
The structure of antrocin (**1**) was elucidated by NMR spectroscopy, mass spectrometry and X-ray crystallography studies. The EI-mass spectrum of **1** indicated a molecular formula of $C_{15}H_{22}O_2$. Its IR absorption band at 1763 cm^{-1} indicates a γ-lactone system [3, 4], and a terminal methylene at 893 cm^{-1}. The above data were indicative of a three-membered ring system.

The ^{13}C NMR and DEPT spectra of **1** showed signals of 15 carbon atoms corresponding to Me ($\times 2$), CH_2 ($\times 7$), CH ($\times 2$), three quaternary carbons and one carbonyl carbon. Irradiation of the methyl group at $\delta 0.93$ (H-13) gave NOE enhancement of $\delta 1.36$ *dd* (H-5), 1.18 *s* (H-14) and 1.21 *m*, $\delta 1.55$ *m* (H-3). Irradiation of the terminal methylene group (H-12) at $\delta 4.83$ and 4.80 gave NOE enhancement of $\delta 2.25$ *m*, 2.35 *m* (H-7) and 2.66 (H-9). Two protons on the lactone's γ-position (H-11) at $\delta 4.14$ (*d*, $J = 9.1$ Hz) and 4.48 (*dd*, $J = 6.8$, 9.1 Hz) coupled to the methine proton (H-9) at $\delta 2.66$ (*d*, $J = 6.8$ Hz).

The ^1H and ^{13}C NMR spectral chemical shifts of **1** were assigned on the basis of 2 D NMR (^1H–^1H COSY, ^{13}C–^1H COSY) and difference NOE techniques (Table 1). A single crystal X-ray diffraction study further confirmed the molecular structure of **1**.

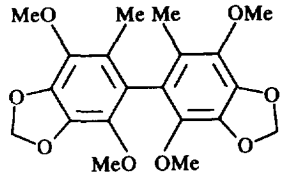
The molecular formula of **2a** was assigned as $C_{10}H_{12}O_4$ by HR-mass spectrometry. In the IR spectrum, bands at 1584 and 1612 cm^{-1} showed that the compound was a benzenoid [5]. The ^1H NMR spectrum showed signals for two methoxy ($\delta 3.84$, 3,88), one methylenedioxy ($\delta 5.93$), one methyl group ($\delta 2.18$) and a single aromatic proton ($\delta 6.30$) [5, 6]. Considering all the spectral evidence, six possible forms (**2a**–**2f**) were possible for





1

	R_1	R_2	R_3	R_4
2a	OMe	H	Me	OMe
2b	H	Me	OMe	OMe
2c	Me	H	OMe	OMe
2d	H	OMe	Me	OMe
2e	Me	OMe	H	OMe
2f	H	OMe	OMe	Me



3

Table 1 ^{1}H and ^{13}C NMR spectral data of **1** ($CDCl_3$ solution, δ value in ppm, J value in Hz)

Atom	^{1}H	^{13}C
1	1.35 *m*, 2,15 *m*	36.8
2	1.50 *m*, 1.80 *m*	18.6
3	1.21 *m*, 1.55 *m*	41.9
4	—	33.2
5	1.36 *m*	46.6
6	1.53 *m*, 1.80 *m*	22.1
7	2.25 *m*, 2.35 *m*	30.3
8	—	146.7
9	2.66 *d* (J = 6.8)	54.1
10	—	48.4
11	4.48 *dd* (J = 6.8, 9.1) 4.14 *d* (J = 9.1)	69.3
12	4.83 *s*, 4.80 *s*	111.1
13	0.93 *s*	33.1
14	1.18*s*	22.3
15	—	178.3

2a. The proposed structure (**2a**) was confirmed by a difference NOE experiment.

Irradiation of the methyl group led to enhancements of the aromatic proton and one methoxy group at δ(3.88), and thus (**2c**-**2f**) can be ruled out. Further, irradiation of the single aromatic proton gave NOE enhancements of the methyl group and the other methoxy group (δ3.84). The above experiment indicated the structure of **2a** to be 4,7-dimethoxy-5-methyl-1,3-benzodioxole.

Compound **3** gave a molecular ion peak in the HR-mass spectrum at m/z 390.1306 corresponding to a molecular formula $C_{20}H_{22}O_8$. In the IR spectrum, it showed the absorption of a benzene ring at 1616 and 1506 cm^{-1}. In the ^{1}H NMR spectrum, one signal of a methylenedioxy at δ5.95, two methoxy groups at δ3.92 and δ3.73 and one methyl group at δ1.78 were observed but there was no aromatic proton. The ^{13}C NMR spectrum also indicated the functional group mentioned above. Comparison of the spectral data of **3** with those of **2** showed similarities. In particular, **3** was a biphenyl of the phenyl of **2**. On the basis of the above data, **3** is proposed to have a structure similar to that of **2**. However, the spectral data alone could not provide a precise structure. Therefore an X-ray analysis was carried out and the structure was determined unequivocally. ORTEP diagrams [7] for the molecular structure are shown in Figs 1 and 2.

The skeleton of **1** consists of two six-membered cyclohexanes, with one ring [C(1), C(5) to C(10)] being chair form and the other being twist–boat form and a five-membered lactone which are fused together with C(5)–C(10) and C(9)–C(10) as the sharing edges. Except the C(15) = O(2) and C(8) = C(12), all bonds in **1** are single bonds. The range of the C–C single bond lengths [1.499(8) to 1.560(8) Å] and the two double bonds [1.196(7) and 1.325(7) Å] are reasonable. The bond length of C(15)–O(1) is significantly smaller than that of C(11)–O(1). This is expected since the bond strength of C_{sp}^2–O is stronger than that of C_{sp}^3–O. Compound **3** is a biphenyl derivative with each phenyl group fused by a heterocyclic five-membered ring and substituted by two

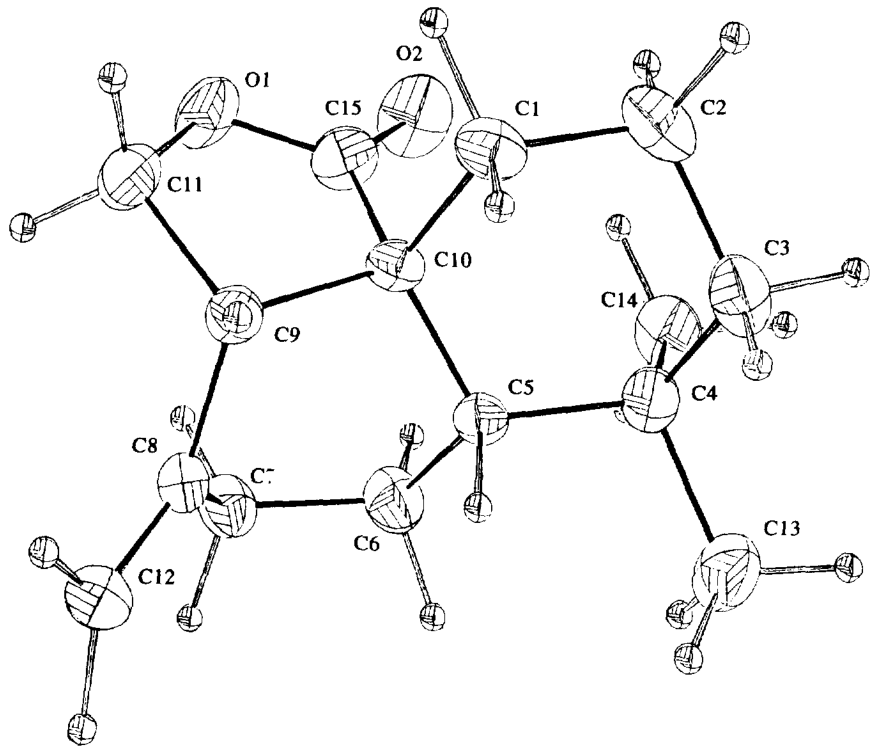


Fig. 1. Molecular structure of 1.

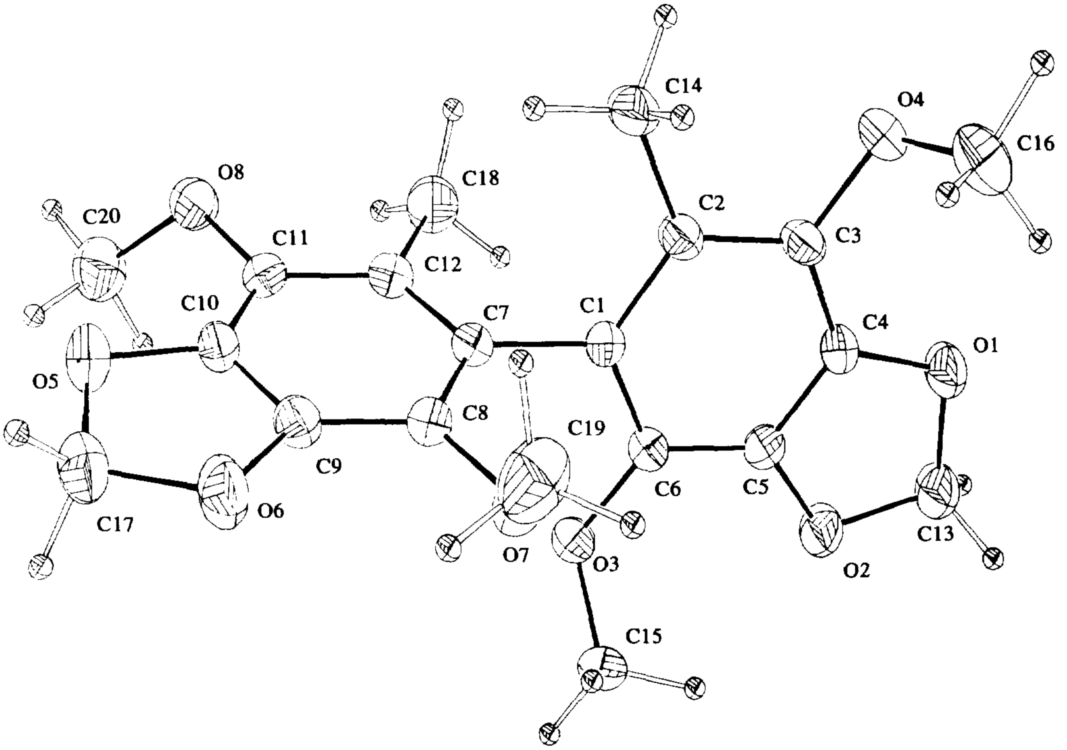


Fig. 2. Molecular structure of 3.

methoxyl groups and a methyl group. The large dihedral angle between the two phenyl groups [81.8(1)°] is indicative of no possible π-interaction between them and the C(1)–C(7) bond is a typical single bond. The nonequality of the C–O bonds in each methoxyl group and in each heterocyclic ring is also due to the different bond strength between C^2_{sp}–O and C^3_{sp}–O bonding. The bond lengths and bond angles of the two phenyl groups are reasonable. No intermolecular contact of structural significance was observed for either compound.

EXPERIMENTAL

General. Mps are uncorr.; IR: KBr disk. ^{1}H and ^{13}C NMR were 400 and 100 MHz, respectively, in $CDCl_3$ soln, with TMS as int. standard. EIMS: 30 eV. HPLC was performed with *n*-hexane–EtOAc on a Si60 (Waters, 6 μm, 7.8 × 300 nm) column employing a refractive index detector at a flow rate of 2.0 ml min^{-1}.

Extraction and sepn. The dry fruit bodies were obtained from *A. cinnamomea* (300 g), growing in Taiwan. They were collected by Ju-Chen Wang, Ling-Chih Co., Taipei, in 1987 and identified by Prof. Chiu-yuan Chien, Institute of Biological Science, National Taiwan Normal University. The fruit bodies were cut into small pieces and refluxed (× 6) with MeOH (2 l) for 5 hr. The conc MeOH extract was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ fr. (70 g) was then chromatographed on a silica gel column (800 g) by stepwise elution with *n*-hexane–EtOAc (7:3), *n*-hexane–EtOAc (1:1), and *n*-hexane–EtOAc (2:3). The *n*-hexane (7:3) elution was chromatographed on a silica gel column repeatedly (*n*-hexane–EtOAc, 10:1) and then sepd by HPLC (*n*-hexane–EtOAc, 15:1) to afford **1** (15 mg), **2a** (40 mg). Compound **3** (30 mg) was crystallized in crude *n*-hexane–EtOAc (7:3) fr.

Antrocin (**1**), (1 β, 4a α, 8a β)-*decahydro-5,5-dimethyl-8-methylene naphthol*[1,8a-c] *furan*-1(3H)-*one*. Crystallized from MeOH as needles, mp 96–98°, $[\alpha]_D^{25}$ − 112° (c = 1.0 $CHCl_3$). IR ν_{max}^{KBr} cm^{-1} : 2957, 1763, 1445, 1366, 1125, 1053, 986, 893; EIMS (30 eV) m/z (rel. int.): 234 $[M]^+$ (6), 219 $[M - Me]^+$ (8), 204 $[M - CH_2O]^+$ (100), 189 $[M - CH_2O - Me]^+$ (7), 175.2 (8), 161 (29), 151 $[M - C_4H_3O_2]^+$ (57), 131 (12), 105 (13), 89 (21). ^{1}H and ^{13}C NMR: see Table 1.

The crystal data of **1** were as follows: a crystal of dimension 0.2 × 0.3 × 0.5 mm^3 was mounted on a Nonius CAD4 diffractometer equipped with Mo radiation (λ = 0.711 Å) and a graphite monochromater. Crystal data: $C_{15}H_{22}O_2$, M_r = 234.34, monoclinic, $P2_1$, a = 6.221(3), b = 16.102(4), c = 7.196(2) Å, β = 113.92(3), V = 659.0(4) Å^3, z = 2, D_c1.181 mg·m^{-3}, F(000) = 255.97. Data collections: $3 \leq 2\theta \leq 50$, using the $\theta/2\ \theta$ scan mode; 1301 total reflections, 1207 unique and 1030 reflections with 1 > 2.5 σ (1). Besides, absorption corrections were made, and the structure was solved by direct method. The final residual: $R = 0.051$, $R_w = 0.046$, GoF = 4.27 for 154 parameters and 1030 reflections. The largest residual electron density was 0.180 eÅ^{-3}. All data reduction and structural refinement were performed by using NRCVAX package [8]. The X-ray data are deposited at the Cambridge Crystallographic Data centre.

Compound **2a**: (4,7-*dimethoxy*-5-*methyl*-1,3-*benzodioxole*). Yellow liquid, IR ν_{max}^{KBr} cm^{-1}: 2952, 2855, 1612, 1584, 1458, 1427, 1346, 1256, 1138, 1065, 1055, 949; HRMS *m/z* 196.0737 $[M]^+$ ($C_{10}H_{12}O_4$ requires: 196.0735); EIMS (20 eV) *m/z* (rel. int.): 196 $[M]^+$ (100), 181 $[M - Me]^+$ (16), 123 (2), 95 (1). ^{1}H NMR ($CDCl_3$): δ6.3 (1H, *s*), 5.93 (2H, *s*), 3.88 (3H, *s*), 3.84 (3H, *s*), 2.18 (3H, *s*). ^{13}C NMR ($CDCl_3$): δ138.8 (C); 138.6 (C); 136.5 (C); 134.6 (C); 134.6 (C); 123.6 (C); 108.8 (CH); 101.4 (CH_2); 59.9 (Me); 56.8; 15.9 (Me).

Compound **3**: (2,2′,5,5′-*tetramethoxy*-3,4,3′4′-*bi-methylenedioxy*-6,6′-*dimethylbiphenyl*). Recrystallized from EtOAc as prism, mp 145–147°; $[\alpha]_D^{25}$ 0° ($CHCl_3$, *c* = 0.2). IR ν_{max}^{KBr} cm^{-1}: 3003, 2937, 2095, 2079, 1616, 1456, 1446, 1419, 1271, 1224, 1122, 1051, 979, 950; EIMS (30 eV) *m/z* (rel. int.): 390 $[M]^+$ 375 $[M - Me]^+$(15), 360 $[M - 2Me]^+$ (8), 345 $[M - 3Me]^+$ (18), 329 $[M - 2Me\ OMe]^+$ (18), 302 (10), 195 $[M - 1/2M]^+$ (15). ^{1}H NMR ($CDCl_3$): δ5.95 (4H, *s*), 3.92 (6H, *s*), 3.73 (6H, *s*), 1.78 (6H, *s*). ^{13}C NMR ($CDCl_3$): δ137.8 (C); 137.2 (C); 136.8 (C); 136.5 (C); 123.0 (C); 101.0 (CH_2); 59.8 (Me); 59.8 (Me); 12.6 (Me).

Compound **3** crystallized in the monoclinic crystal system ($P2_{1/C}$) with $a = 11.226(6)$, $b = 10.823(2)$, $c = 15.702(2)$ and $\beta = 99.58(3)$. A prismatic crystal (0.6 × 0.6 × 0.7 mm^3) was selected and mounted on a Nonius CAD4 diffractometer. Reflections (3438 total) were measured in the range of $3 \leqq \theta \leqq 50°$, using the $\theta/2\ \theta$ scan mode. 3305 unique and 2470 of which were assumed as observed applying the condition $1 > 2.5\sigma$ (1). The final residual: $R = 0.056$, $R_w = 0.58$, GoF = 6.41 for 254 parameters and 2470 reflections. The largest residual electron density was 0.200 eÅ^{-3}.

Acknowledgements—The authors thank the National Science Council of the Republic of China for financial support (NSC84-2113-M-003-002).

REFERENCES

1. Chang, T.-T. and Chou, W.-N. (1995) *Mycol. Res.*, in press.
2. Mu, Z. and Su, Q. (1990) *Acta Botanica Yunnanco* **12**, 395.
3. Lopes, L. M. S., Yoshida, M. and Gottlieb, O. R. (1983) *Phytochemistry* **22**, 1516.
4. Kioy, D., Gray, A. I. and Waterman, P. G. (1990) *Phytochemistry* **29**, 3535.
5. Atal, C. K., Moza, P. N. and Pelter, A. (1968) *Tetrahedron Letters* **11**, 1397.
6. Burke, B. and Nair, M. (1986) *Phytochemistry* **25**, 1427.
7. Johnson, C. K. (1965) *ORTEP*, Report ORNL-3794, Oak Ridge National Laboratory, TN, U.S.A.
8. Larson, A. C., Lee, F. L., Le Page, Y., Webster, M., Charland, J.-P. and Gabe, E. J. (1990) *NRCVAX Crystal Structure System*, Chemistry Division, NRC, Ottawa, Canada.