Phytochemistry, Vol. 30, No. 7, pp. 2193–2195, 1991
Printed in Great Britain.

0031–9422/91 $3.00+0.00

BIOACTIVE NEOLIGNANS FROM THE LEAVES OF *MAGNOLIA VIRGINIANA**

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(*Received in revised form* 3 *January* 1991)

Key Word Index—*Magnolia virginiana*; magnoliaceae; leaves; magnolol; 4,4′-diallyl-2′3′-dihydroxybiphenyl ether; 3,5′-diallyl-2′-hydroxy-4′-methoxybiphenyl; anti-fungal; anti-bacterial; insecticidal.

Abstract—A novel biphenyl ether, 4,4′-diallyl-2,3′-dihydroxybiphenyl ether, 3,5′-diallyl-2′-hydroxy-4-methoxybiphenyl and 5,5′-diallyl-2,2′-dihydroxybiphenyl (magnolol) were isolated from the leaves of *Magnolia virginiana* and characterized by spectral and chemical means. All three compounds and their methoxy analogues were very toxic to brine shrimp and mosquito larvae and showed strong anti-fungal and anti-bacterial activities.

INTRODUCTION

The family Magnoliaceae figures prominently in the host plant patterns of certain insect groups [1]. To identify ecologically significant plant compounds from members of this family, we have been examining the phytochemistry of *Magnolia virginiana* L. This tree is native to the eastern and southeastern United States and has been introduced to other parts of the world as an ornamental [2]. A number of *Magnolia* species have been extensively examined for compounds possessing pharmacological, antimicrobial, and pesticidal activity [3–7]. Antimicrobial activity of phenolic constituents of *M. grandiflora* L. has been reported earlier [6]. Other species of *Magnolia* investigated for antimicrobial and pharmacologically active compounds are *M. officinalis*, *M. liliflora* and *M. obovata* [3–5, 7]. Similar antimicrobial neolignans have also been isolated from the roots of *Sassafras randaiense* [8]. 4′,5-Diallyl-2-hydroxy-3-methoxybiphenyl ether has been reported from the bark of *M. henryi* [9] and a monohydroxy ether, 4′,5-diallyl-2-hydroxybiphenyl ether from *S. randaiense* [8]. Magnolol (**4**) was previously isolated from the seeds of *M. grandiflora* [9] as well as from the bark of *M. henryi* [10]. Our anti-microbial and insecticidal bioassay-directed work on *M. virginiana* leaves resulted in the characterization of three active compounds, **1**, **4** and **6**.

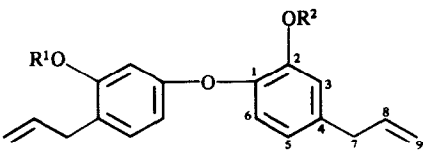
RESULTS AND DISCUSSION

The percentage yield of compounds **1**, **4** and **6** isolated from the dried plant material were 1.07, 0.78 and 0.30, respectively. Centrifugal partition chromatography (CPC) of the crude hexane extract resulted in two fractions, I and II, which contained the biologically active compounds **1**, **4** and **6**, were free of contaminants. This technique is a countercurrent, liquid–liquid partition chromatography which utilizes centrifugal force to immobilize the stationary liquid phase. CPC was proved to be better, less expensive and efficient method compared to the traditional use of column or flash-column chromatography for the initial purification.

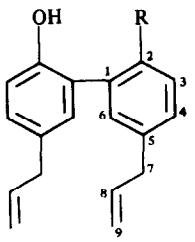
Comparison of the ^{1}H and ^{13}C NMR chemical shifts with the reported values confirmed the identity of compounds **4** and **6** as magnolol [11] and 4-methoxyhonokiol [9], respectively. The presence of two phenolic groups in **4** was confirmed by methylation using dimethyl sulphate [12] and NMR spectral data but methylation with diazomethane afforded exclusively a monomethyl ether, **5** and was confirmed by spectral analysis. Additionally, methylation of one of the phenolic groups in **4** did not affect the chemical shifts of the aromatic ring protons or the two allyl groups in the molecule.

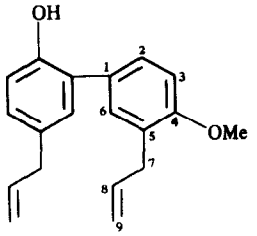
The presence of two phenolic groups in **1** was confirmed by methylation, mass spectral and NMR data and the third oxygen functionality in the molecule was assigned to an ether. There is no carbonyl group in **1** as evidenced by IR and ^{13}C NMR data. It is interesting to note that methylation of **1** with diazomethane gave a mixture of mono- and dimethoxy products. However, **1** produced only a dimethoxy derivative by reacting with dimethyl sulphate in acetone–K_2CO_3 [12]. If both hydroxyls in **1** were oriented as in the case of **4**, then diazomethane methylation of **1** should have produced only a monomethoxy product. In addition, the ^{13}C NMR data did not show completely symmetrical signals for **1** compared to the nine signals for 18 carbons in **4**. The ^{1}H NMR signals at δ6.35 and 6.68 for **1**, assigned to H-2′ and H-3, respectively, suggested the absence of such symmetry in **1**. The ^{13}C NMR of **1** gave 16 signals and the chemical shift at δ129.53 and 117.51, integrated for two carbons each, were assigned to 5,5′ and 6,6′ carbons, respectively. Although, biphenyl ethers of this nature have been reported from *S. randaiense* [8], **1** is a new member of this class of biphenyl ethers. To our knowledge, this is the first report of **1** as a natural product from *Magnolia* species.

*Contribution from the Michigan State University Agriculture Experiment Station.



1 R^1 = OH, R^2 = OH
2 R^1 = OMe, R^2 = OH
3 R^1 = OMe, R^2 = OMe





4 R = OH
5 R = OMe

6

Antimicrobial activities of magnolol and related neolignans were reported earlier [6]. We have evaluated the biological activities of all six compounds, **1–6**, for fungicidal, bacteriocidal, insecticidal and nematocidal properties. The biphenyl ether, **1**, and its methylated products showed slightly better broad spectrum activity (Table 1) than **4–6**. All test compounds showed similar toxicity to mosquito larvae and brine shrimp. At 100 ppm concentration, **1–6** gave 100% mortality for both mosquito and brine shrimp larvae within 30 min and was similar to the control valinomicin. At 10 ppm concentration, **1** and **6** gave 100% mortality to both test species with 2 hr and **3–5** produced the same result in 12 hr. None of the test compounds showed any activity towards nematodes. This is the first report of these neolignans showing insecticidal activity.

EXPERIMENTAL

General. Mps: uncorr. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.45 MHz): $CDCl_3$. MS (70 eV, EI); UV: MeOH; IR: $CHCl_3$. Prep. TLC and TLC were carried out on silica gel and the solvent systems were Et_2O–hexane (1:4) or Me_2CO–hexane (1:4). Initial purification of the hexane extract of the plant material was carried out by centrifugal partition chromatography (CPC) using the lower layer of the solvent system hexane–MeCN–EtOAc–H_2O (8:7:5:1) as the mobile phase.

Antifungal bioassay. Known amounts of the pure test compounds were dissolved in DMSO and serial dilutions prepared in the same solvent. A 20 μl aliquot of each soln was mixed with 2 ml of Emmons liquid medium seeded with *ca* 2 × 10^3 CFU ml^{-1} of the test organism. The inoculated tubes were vortexed and incubated at 26°. Similarly, inoculated tubes without test compounds served as controls. Depending on the growth characteristics of the test species, results were recorded after 2–4 days. The lowest concentration of the test compound that totally inhibited growth of test organism was recorded as the MIC for that species.

Antibacterial assay. The antibacterial activity of all test compounds was evaluated by the same procedure as in the antifungal assay except that Mueller–Hinton broth was used as the medium and the test organism inoculum was 10^4 CFU each. The inoculated tubes containing test compounds and control were incubated at 37° for 24 hr and scored for growth of each test organism. The MIC for each species represents the lowest concentration of the test compound at which complete inhibition of growth occurred.

Insecticidal assay. The bioassay for insecticidal properties was conducted on 4th instar mosquito larvae, *Aedes aegypti*, reared

Table 1. Minimum inhibitory concentrations (MIC) of compounds **1–6**

Organism	Compounds (μg ml^{-1})					
	1	**2**	**3**	**4**	**5**	**6**
Fungi						
Candida albicans	10	10	10	50	25	25
Aspergillus flavus	10	10	10	75	50	50
Gleosporum sp.	25	25	25	25	25	25
Rhizoctonia sp.	25	25	25	25	25	25
Bacteria						
Streptococcus aureus	10	10	10	25	15	15
Staphylococcus epidermidis	10	10	10	20	10	10
Escherichia coli	20	20	20	50	25	25
Insect*						
Aedes aegypti (Mosquito larvae)	10	10	10	10	10	10
Crustacea*						
Artemia salina (Brine shrimp)	1	1	1	1	1	1
Nematode*						
Caenorhabditis elegaus	NA	NA	NA	NA	NA	NA
Panagrellus redivivus (axenic)	NA	NA	NA	NA	NA	NA

NA = not active.
*Activity measured at 12 hr.

from the mosquito eggs (University of Davis California Straw, courtesy of Drs Fumio Matsumura and David Grant) and on brine shrimp, *Artemia salina* Leach (obtained from store). For mosquitocidal assay, 10 larvae were placed in 975 μl dist. H_2O and 25 μl of test compounds in DMSO added and left at room temp. The number of dead larvae was recorded at 2, 4 and 24 hr intervals. The control tube containing 10 larvae received 25 μl of DMSO alone and mortality was recorded as in the case of test compounds.

Brine shrimp assay. The eggs of brine shrimp was placed in artificial sea water prepared by dissolving 38 g of sea salt l^{-1} H_2O and left at 24° for 48 hr. The larvae were then transferred into test tubes containing sea water and the experiment was conducted as with the mosquito larvae.

Isolation of compounds **1**, **4** *and* **6**. *Magnolia virginiana* trees were obtained from Herren Nursery, Florida division of Forestry and grown in the green house of Michigan State University for 1 year. Fresh leaves were collected from potted greenhouse trees, freeze-dried, and ground to a powder. The powdered foliage (435 g) was sequentially extracted with hexane (6 l), EtOAc (6 l) and MeOH (2 l), and subsequent removal of solvent *in vacuo* afforded 36.9 g, 16.9 g and 54.34 g of residue, respectively. The hexane fr. (12.78 g) was initially purified by CPC (3.8 ml flow rate at 900 rpm) and two frs at R_fs 9.6 (7.37 g), fr. I, and 12.6 (0.915 g), fr. II, were collected and further purified by column and prep. TLC. Final purification of fr. I (0.443 g) afforded compounds **1** (147.8 mg) and **4** (100 mg) which were confirmed to be pure by TLC. Similar purification of fr. II (0.465 g) yielded **6** (207.6 mg).

4,4′-*Diallyl*-2,3′-*dihydroxybiphenyl ether* (**1**). Pale yellow oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3494, 1602, 1504, 1213, 1822, 992, 914, 844; UV λ_{max}^{MeOH}: 290 and 270 nm (ε 6210 and 29 900) and by the addition of KOH in MeOH, 319, 226 and 206 nm (ε 7780, 28 200 and 24 300); HRMS *m/z* 282.1257 (calcd 282.1261 for $C_{18}H_{18}O_3$, $[M]^+$, 100%), 241 (5), 213 (3), 200 (3), 117 (5), 91 (5); ^{1}H NMR: δ3.23 (2H, *d*, $J=5$ Hz, CH_2 benzylic), 3.40 (2H, *d*, $J=5$ Hz, CH_2, benzylic), 5.10 (4H, *m*, $2\times CH_2$ vinyl), 5.70 (1H, *br s*, exchanged with D_2O, phenol), 5.75 (1H, *br s*, exchanged with D_2O, phenol), 5.95 (2H, *m*, $2\times$CH vinyl), 6.35 (1H, *d*, $J=2$ Hz, H-2′), 6.65 (1H, *d*, $J=2$ Hz, H-3), 6.95 (2H, *dd*, $J=9$, 2 Hz, H-6, H-6′), 7.17 (2H, *dd*, $J=9$, 2 Hz, H-5, H-5′); ^{13}C NMR: δ144.52 (C-1), 134.90 (C-2), 110.91 (C-3), 132.65 (C-4), 129.53 (C-5, 5′), 117.51 (C-6, 6′), 38.96 (C-7), 137.09 (C-8), 115.51 (C-9), 143.51 (C-1′), 110.41 (C-2′), 154.82 (C-3′), 132.22 (C-4′), 38.74 (C-7′), 136.93 (C-8′), 115.40 (C-9′), 55.47 (OMe).

CH_2N_2 *methylation of compound* **1**. Compound **1** (100 mg) was dissolved in Et_2O (10 ml) and mixed with CH_2N_2 saturated in ether (10 ml). The resulting yellow solution was kept at room temp. (18 hr) and evapd to dryness. A TLC analysis of this product indicated two compounds and separation was achieved on TLC using hexane–Me_2CO (4:1). The high R_f compound **2**, dimethyl ether of **1**; EI-MS *m/z* 310 ($[M]^+$, 100); ^{1}H NMR: δ3.25 (2H, *d*, $J=5$ Hz, CH_2 benzylic), 3.35 (2H, *d*, $J=5$ Hz, CH_2 benzylic), 3.80 (3H, *s*, OMe), 3.90 (3H, *s*, OMe), 5.10 (4H, *m*, $2\times CH_2$ vinyl), 5.95 (2H, *m*, $2\times$CH vinyl), 6.43 (1H, *d*, $J=2$ Hz, H-2′), 6.60 (1H, *d*, $J=2$ Hz, H-3), 6.90 (2H, *dd*, $J=9$, 2 Hz, H-6, H-6′), 7.15 (2H, *dd*, $J=9$, 2 Hz, H-5, H-5′). Low R_f compound **3**, monomethyl ether; EI-MS *m/z* 296.0 ($[M]^+$, 100); ^{1}H NMR (300 MHz, $CDCl_3$): δ3.25 (2H, *d*, $J=5$ Hz, CH_2 benzylic), 3.40 (2H, *d*, $J=5$ Hz, CH_2 benzylic), 3.90 (3H, *s*, OMe), 5.10 (4H, *m*, $2\times CH_2$ vinyl), 5.80 (1H, *s*, exch. with D_2O, phenol), 5.95 (2H, *m*, $2\times$CH vinyl), 6.35 (1H, *d*, $J=2$ Hz, H-2), 6.60 (1H, *d*, $J=2$ Hz, H-3), 6.90 (2H, *dd*, $J=9$, 2 Hz, H-6, H-6′), 7.15 (2H, *dd*, $J=9$, 2 Hz, H-5, H-5′).

5,5′-*Diallyl*-2,2′-*dihydroxybiphenyl* (**4**). Needle-like crystals, mp 101–102°; IR $\nu_{max}^{CHCl_3}$: 3372, 2360, 1638, 1496, 1417, 1722, 914, 821; UV λ_{max}^{MeOH} 275 and 204 nm (ε 5440 and 49 500) and by the addition of KOH in MeOH 279 and 223 nm (ε 6420 and 29 300); HRMS *m/z* 266.1313 ($[M]^+$, calcd 266.1335 for $C_{18}H_{18}O_2$, 100%), 247 (10), 237 (18), 225 (16), 207 (10), 197 (17), 184 (15), 165 (5), 91 (5); ^{1}H NMR: δ3.39 (4H, *d*, $J=5$ Hz, $2\times CH_2$ benzylic), 5.10 (4H, *m*, $2\times CH_2$ vinyl), 6.00 (2H, *m*, $2\times$CH vinyl), 6.20 (2H, *br s* exch. with D_2O, phenol), 6.90 (2H, *dd*, $J=9$, 2 Hz, H-3, H-3′), 7.12 (4H, *d* and *dd*, overlapped $J=9$, H-4, H-4′ and H-6, H-6′); ^{13}C NMR: δ144.52 (C-1, C-1′), 134.90 (C-2, C-2′), 110.91 (C-3, C-3′), 132.65 (C-4, C-4′), 129.53 (C-5, C-5′), 117.51 (C-6, C-6′), 38.96 (C-7, C-7′), 137.09 (C-8, C-8′), 115.51 (C-9, C-9′).

CH_2N_2 *methylation of compound* (**4**). Methylation of **4** with CH_2N_2 carried out as in the case of **1**. The single product obtained, **5**, a monomethylether, gave EI-MS *m/z* 280 ($[M]^+$, 100); ^{1}H NMR: δ3.40 (4H, overlapped *d*, $J=5$ Hz, $2\times CH_2$ benzylic), 3.90 (3H, *s*, OMe), 5.10 (4H, *m*, $2\times CH_2$ vinyl), 6.00 (2H, *m*, $2\times$CH vinyl), 6.25 (1H, *s*, exch. with D_2O, phenol), 7.00 (2H, *dd*, $J=9$, 2 Hz, H-3, H-3′), 7.20 (4H, *m*, H-4, H-4′ and H-6, H-6′).

3,5′-*Diallyl*-2′-*hydroxy*-4-*methoxybiphenyl* (**6**). Pale yellow oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350 (OH), 1640 (olefinic C=C), 1605 (aromatic C=C); ^{1}H NMR: δ3.35 (2H, *d*, $J=6$ Hz, benzylic CH_2), 3.45 (2H, *d*, $J=6$ Hz, benzylic CH_2), 3.90 (3H, *s*, OMe), 5.10 (4H, *m*, vinyl $CH_2\times 2$), 5.25 (1H, *br s*, exch. with D_2O, phenolic), 6.01 (2H, *m*, olefinic CH × 2), 6.95 (1H, *dd*, $J=8$, 1.5 Hz, H-3′), 6.97 (1H, *dd*, $J=8$, 1.5 Hz, H-5), 7.05 (2H, *m*, H-2, H-6′), 7.23 (1H, *dd*, $J=8.5$, 1.6 Hz, H-6), 7.30 (1H, *dd*, $J=8.5$, 1.6 Hz, H-4′); ^{13}C NMR: (δ129.09 (C-1), 130.67 (C-2), 128.67 (C-3), 156.94 (C-4), 115.53 (C-5), 127.86 (C-6), 34.22 (C-7), 136.48 (C-8), 115.47 (C-9), 127.83 (C-7′), 150.79 (C-2′), 110.88 (C-3′), 129.64 (C-4′), 132.09 (C-5′), 130.46 (C-6′), 39.36 (C-7′), 137.77 (C-8′), 115.77 (C-9′), 55.47 (OMe). EI-MS *m/z* (rel. int.) 280 ($[M]^+$, 100), 251 (20), 224 (15), 198 (20), 181 (5), 165 (5), 84 (60), 831 (90).

Acknowledgements—The authors are grateful to Dr Long D. Le (Max T. Rogers NMR facility, Department of Chemistry, Michigan State University) for the NMR spectra. This work was supported in part by the grants from USDA (87-CRCR-1-2581), Michigan State University Foundation, and Michigan State Agricultural Research Station Project 8051.

REFERENCES

1. Scriber, J. M. (1986) *Oecologia* **71**, 94.
2. Little, E. L. (1980) *The Audubon Society Field Guide to North American Trees (Eastern Region)*, p. 714. Alfred A. Knopf, New York.
3. Rao, K. V. and Davis, T. L. (1982) *J. Nat. Prod.* **45**, 283.
4. Kimura, I., Kimura, M., Yoshizaki, M., Yanada, K., Kadota, S. and Kikuchi, T. (1983) *Planta Med.* **48**, 43.
5. Kamikado, T., Chang, C.-F., Murakoshi, S., Sakurai, A. and Tamura, S. (1975) *Agric. Biol. Chem.* **39**, 833.
6. Clark, A. M., El-Feraly, F. S. and Li, W.-S. (1980) *J. Pharm. Sci.* **70**, 951.
7. Watanabe, K. (1985) *Yaoxue Tongbao*, **20**, 522.
8. El-Feraly, F. S., Cheatham, S. F. and Breedlove, R. L. (1983) *J. Nat. Prod.* **46**, 493.
9. El-Feraly, F. S. and Li, W. S. (1978) *Lloydia* **41**, 442.
10. Fujita, M., Hideji, I. and Sashida, Y. (1973) *Yakugaku Zasshi*, **93**, 429.
11. Kijjoa, A., Pinto, M. M., Tantisewie, B. and Herz, W. (1989) *Phytochemistry* **28**, 1284.
12. Nair, M. G., Sommerville, J. and Burke, B. A. (1989) *Phytochemistry* **28**, 654.