(2) A. W. Pruitt, J. S. Winkel, and P. G. Dayton, *Clin. Pharmacol. Ther.*, **21**, 610 (1977).
(3) W. I. Baba, G. R. Tudhope, and G. M. Wilson, *Br. Med. J.*, **2**, 756 (1962).
(4) S. T. Kau, B. V. Rama Sastry, J. D. Alvin, and M. T. Bush, *Drug Metab. Dispos.*, **3**, 345 (1975).
(5) B. Grebian, H. E. Geissler, and E. Mutschler, *Arzneim.-Forsch.*, **26**, 2125 (1976).
(6) P. G. Dayton, A. W. Pruitt, J. L. McNay, and J. Steinhorst, *Neuropharmacology*, **11**, 435 (1972).
(7) S. Sved, J. A. A. Sertie, and I. J. McGilveray, *J. Chromatogr.*, **162**, 474 (1979).
(8) R. R. Brodie, L. F. Chasseaud, T. Taylor, and L. M. Walmsley, *ibid.*, **164**, 527 (1979).

ACKNOWLEDGMENTS

Presented in part at the APhA Academy of Pharmaceutical Sciences, Washington, D.C., meeting, April 1980.

Supported in part by a grant-in-aid from the American Heart Association and by funds contributed in part by the Texas Affiliate.

The authors express their gratitude to Smith Kline and French Laboratories and to Rohm & Haas for authentic samples of triamterene and 6-*p*-hydroxytriamterene.

Antimicrobial Activity of Phenolic Constituents of *Magnolia grandiflora* L.

ALICE M. CLARKˣ, **FAROUK S. EL-FERALY**, and **WEN-SHYONG LI** *

Received December 8, 1980, from the *Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677.* Accepted for publication January 14, 1981. *Present address: Taiwan Provincial Pingtung Institute of Agriculture, Pingtung, Taiwan, Republic of China.

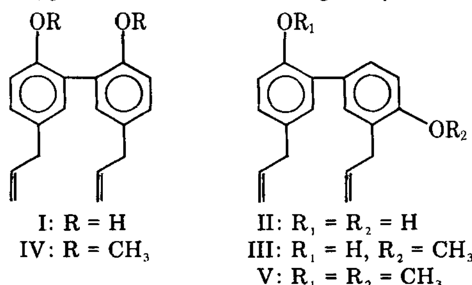
Abstract □ Three phenolic constituents of *Magnolia grandiflora* L. were shown to possess significant antimicrobial activity using an agar well diffusion assay. Magnolol, honokiol, and 3,5′-diallyl-2′-hydroxy-4-methoxybiphenyl exhibited significant activity against Gram-positive and acid-fast bacteria and fungi. The minimum inhibitory concentrations were determined for each compound using a twofold serial dilution assay.

Keyphrases □ *Magnolia grandiflora* L.—antimicrobial activity of phenolic constituents, agar well diffusion assay □ Magnolol—phenolic constituent of *Magnolia grandiflora* L., antimicrobial activity □ Honokiol—phenolic constituent of *Magnolia grandiflora* L., antimicrobial activity □ 3,5′-Diallyl-2′-hydroxy-4-methoxybiphenyl—phenolic constituent of *Magnolia grandiflora* L., antimicrobial activity □ Antimicrobial activity—phenolic constituents of *Magnolia grandiflora* L., agar well diffusion assay

The search for new antibiotics is no longer restricted primarily to microbial products. Recently, constituents of higher plants have exhibited significant antimicrobial activity (1–4). The isolation and identification of magnolol (I), honokiol (II), and 3,5′-diallyl-2′-hydroxy-4-methoxybiphenyl (III) as phenolic constituents of the seeds of *Magnolia grandiflora* L. were reported (5). All three compounds have significant antifungal and antibacterial activity, and the details of this antimicrobial activity are described here.

RESULTS AND DISCUSSION

Magnolol (I), honokiol (II), and 3,5′-diallyl-2′-hydroxy-4-methoxybiphenyl (III), phenolic constituents of *M. grandiflora* L. (5), showed



significant antimicrobial activity against Gram-positive bacteria, an acid-fast bacterium, and yeast-like and filamentous fungi. All three compounds were tested qualitatively for activity using an agar well diffusion assay (Table I). The corresponding dimethyl ethers, IV and V, showed no significant activity.

Streptomycin sulfate and amphotericin B also were tested to serve as standards for comparison. All three compounds (I–III) had activity comparable to the standards under the same test conditions.

The minimum inhibitory concentration (MIC) of each active compound was determined using a twofold serial dilution assay (Table II). All three compounds apparently were considerably more active than amphotericin B against *Trichophyton mentagrophytes* but not as active against *Candida albicans* and *Saccharomyces cerevisiae.* Methylation of one phenolic group of II to give III resulted in the loss of activity against *Aspergillus niger* and *C. albicans* but not against other fungi or bacteria. In fact, III was somewhat more active against *Staphylococcus aureus, Bacillus subtilis,* and *Mycobacterium smegmatis* than the dihydroxy compounds I and II. All three compounds possessed activity comparable to, or better than, streptomycin sulfate against *B. subtilis, S. aureus,* and *M. smegmatis.* Compounds I–III were also tested for activity against Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), but none showed any activity.

EXPERIMENTAL

Qualitative Antimicrobial Screening—All compounds were tested for activity against the following microorganisms: *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 15442), *Mycobacterium smegmatis* (ATCC 607), *Candida albicans* (ATCC 10231), *Saccharomyces cerevisiae* (ATCC 9763), *Aspergillus niger* (ATCC 16888), and *Trichophyton mentagrophytes* (ATCC 9972). Routine qualitative screening of compounds for antimicrobial activity was accomplished as previously described (1) except for the following modifications: plates for the assay were prepared by dispensing 25 ml of sterile agar medium into 100 × 15-mm sterile petri dishes; and using the quadrant streak method, the sterile agar plates were streaked with a dilution of the test organism (1 ml of broth culture in 9 ml of sterile water).

Antimicrobial activity was recorded as the width (in millimeters) of the inhibition zone measured from the edge of the agar well to the edge of the inhibition zone.

Quantitative Antimicrobial Assay—For compounds that showed significant activity in the qualitative screen, the MIC values were determined using the twofold serial dilution technique previously described (1). All compounds were initially tested using a concentration of 100 μg/ml in the first tube. After preliminary evaluation to determine the range of the MIC value, the concentrations in the first tube were de-

0022-3549/81/0800-0951$01.00/0
© 1981, American Pharmaceutical Association

Table I—Antimicrobial Activity of Phenolic Constituents of *M. grandiflora* L.

Compound	Zone Diameter, mm						
	B. subtilis	*S. aureus*	*M. smegmatis*	*C. albicans*	*S. cerevisiae*	*A. niger*	*T. mentagrophytes*
I	8	7	15	6	12	6	17
II	9	10	12	6	12	8	20
III	7	6	13	2	15	1	15
IV	1	2	—	—	4	—	4
V	—	—	—	—	3	—	3
Streptomycin sulfate	10	7	17	NT[a]	NT	NT	NT
Amphotericin B	NT	NT	NT	4	5	3	4

[a] Not tested.

Table II—Minimum Inhibitory Concentrations (Micrograms per Milliliter) of Phenolic Constituents of *M. grandiflora* L.[a]

Compound	*B. subtilis*	*S. aureus*	*M. smegmatis*	*C. albicans*	*S. cerevisiae*	*A. niger*	*T. mentagrophytes*
I	5 (5)	5 (5)	5 (5)	30 (30)	10 (10)	30 (30)	2.5 (2.5)
II	5 (5)	10 (10)	7.5 (7.5)	30 (30)	10 (10)	30 (30)	2.5 (2.5)
III	2.5 (2.5)	2.5 (2.5)	2.5 (2.5)	NT[b]	10 (10)	NT	1.25 (1.25)
Streptomycin sulfate	10 (10)	10 (10)	2.5 (1.25)	NT	NT	NT	NT
Amphotericin B	NT	NT	NT	5 (5)	2.5 (2.5)	30 (30)	15 (15)

[a] Numbers in parentheses refer to values obtained on duplicate testing. [b] Not tested.

creased to either 30 or 60 μg/ml.

The MIC was taken as the lowest concentration that inhibited growth after 24 or 48 hr of incubation. Tubes inoculated with *B. subtilis* and *S. aureus* were incubated at 37° for 24 hr, while tubes inoculated with *M. smegmatis* were incubated at 37° for 48 hr. Tubes inoculated with fungi and yeasts were incubated at 30° for 48 hr. Streptomycin sulfate[1] and amphotericin B[2] were used as standard antibiotics for comparison with the phenolic compounds.

REFERENCES

(1) C. D. Hufford, M. J. Funderburk, J. M. Morgan, and L. W. Robertson, *J. Pharm. Sci.*, **64**, 789 (1975).

(2) C. D. Hufford and W. L. Laswell, Jr., *Lloydia*, **41**, 156 (1978).

(3) L. A. Mitscher and A. Al-Shamma, *Annu. Rep. Med. Chem.*, **15**, 255 (1980).

(4) L. A. Mitscher, Y. H. Park, and D. Clark, *J. Natl. Prod.*, **43**, 259 (1980).

(5) F. S. El-Feraly and W.-S. Li, *Lloydia*, **41**, 442 (1978).

[1] Chas. Pfizer & Co., Groton, Conn.
[2] Calbiochem, San Diego, Calif.

ACKNOWLEDGMENTS

Supported in part by the Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi.

The authors are grateful to Ms. Sue Johnson for technical assistance with the antimicrobial assay.

Preparation and Antidiabetic Activity of Cyclic Sulfonylthiourea Derivatives

RAAFAT SOLIMAN *x, HASSAN M. FEID-ALLAH §, and HOSNY F. MOHAMED ‡

Received September 29, 1980, from the **Department of Pharmaceutical Chemistry and the ‡Department of Pharmacology, Faculty of Pharmacy, and the §Department of Chemistry, Faculty of Science, University of Alexandria, Alexandria, Egypt.* Accepted for publication January 21, 1981.

Abstract □ 3-Substituted 5-methyl-1-{*p*-[(3,5-dimethyl)pyrazol-1-yl]-, 5-methyl-1-{*p*-[(5-methyl-3-carboxy)pyrazol-1-yl]-, 1-{*p*-[(3-methyl-5-phenyl)pyrazol-1-yl]-, and 1-{*p*-[(3-methyl-4-bromo-5-phenyl)pyrazol-1-yl]benzenesulfonyl}-2-thiohydantoin and their 5-methyl-2-thiohydantoin and 5,6-dihydro-4(3*H*)-oxo-2(1*H*)-pyrimidinethione derivatives were prepared for evaluation as hypoglycemic agents. Biological testing showed that some of these compounds possessed antidiabetic activity.

Keyphrases □ Cyclic sulfonylthiourea derivatives—preparation and evaluation for antidiabetic activity, IR and PMR spectroscopy □ IR spectroscopy—identification of cyclic sulfonylthiourea derivatives, antidiabetic activity in mice □ Antidiabetic activity—synthesis and evaluation of cyclic sulfonylthiourea derivatives, identification by IR spectroscopy

In spite of the low hypoglycemic effect of substituted pyrazolesulfonylthiourea derivatives (1–3), their cyclic thio analogs showed potent antidiabetic activity (1). This finding initiated the synthesis of new cyclic pyrazolesulfonylthio analogs[1] for the evaluation of their antidiabetic effect.

EXPERIMENTAL[2]

Substituted *p*-[(3,5-dimethyl)pyrazol-1-yl]-, *p*-[(5-methyl-3-carboxy)pyrazol-1-yl]-, *p*-[(3-methyl-5-phenyl)pyrazol-1-yl]-, and *p*-[(3-methyl-4-bromo-5-phenyl)pyrazol-1-yl]benzenesulfonylthiourea derivatives were prepared by the treatment of their corresponding *p*-sulfamylphenylpyrazole derivatives with the appropriate isothiocyanates.

[1] Application for a patent was made for compounds described in this report.
[2] Melting points were determined on a Kofler block and are uncorrected. IR spectra were determined as Nujol mulls with a Beckman IR-4210 spectrometer. PMR spectra was recorded on a Varian A-60 A spectrometer. Microanalyses were performed by the Microanalytical Unit, Faculty of Science, University of Cairo, Cairo, Egypt.

0022-3549/81/0800-0952$01.00/0
© 1981, American Pharmaceutical Association