

All phenolics were identified by standard procedures and hydrolytic data, as well as by authentic sample comparison and color reaction procedures (4-7). This is the first report of the occurrence of xanthone-C-glycosides from the genus *Rhynchosia*. Xanthenes have been found in a limited number of families. They always occur in the Guttiferae and Gentianaceae (8) and are considered to be characteristic of these plants; xanthone-C-glycosides are known to occur in two genera, *Hedyrarium* and *Peltoporum* of the Leguminosae and in ferns (9). Full details of the isolation and identification of the compounds are available on request to the senior author.

ACKNOWLEDGMENTS

One of the authors (PR) is grateful to UGC New Delhi, for financial assistance. Our thanks are due to Prof. M. Aritomi, Kumamoto University, Japan, and Prof. a. Jacot-guillarmod, Universite de Neuchatel, Switzerland, for authentic samples of mangiferin and isomangiferin.

LITERATURE CITED

1. D. Adinarayana, D. Gunasekar, and P. Ramachandraiah, *Curr. Sci.*, **48**, 727 (1979).
2. D. Adinarayana, D. Gunasekar, P. Ramachandraiah, O. Seligmann, and H. Wagner, *Phytochemistry*, **19**, 478 (1980).
3. D. Adinarayana, P. Ramachandraiah, O. Seligmann, and H. Wagner, *Phytochemistry*, **20**, 2058 (1981).
4. J.B. Harborne, "Phytochemical Methods," London: Chapman and Hall, 1973, pp. 52-59.
5. K. Paech and M.V. Tracey, "Modern Methods of Plant Analysis," Vol. III, New York: Springer Verlag, 1955, pp. 66-79.
6. T.J. Mabry, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," New York: Springer Verlag, 1970, pp. 41-53, 261-273.
7. J.B. Harborne, T.J. Mabry, and H. Mabry, "The Flavonoids," London: Chapman and Hall, 1975, pp. 21-30.
8. K. Hostettmann and H. Wagner, *Phytochemistry*, **16**, 821 (1977).
9. P.M. Richardson, *Biochem. Syst. Ecol.*, **11**, 371 (1983).

Received 11 June 1984

METHYL β -ORCINOLCARBOXYLATE AND DEPSIDES FROM *PARMELIA FURFURACEA*

S. CACCAMESE, R.M. TOSCANO,

Department of Chemical Sciences, University of Catania, Catania, Italy

F. BELLESIA, and A. PINETTI

Institute of Organic Chemistry, University of Modena, Modena, Italy

Species of the genus *Parmelia* have been shown to produce antimicrobial constituents (1), and in the case of *Parmelia furfuracea* (L.) Ach., a common conifer lichen, extracts have been utilized to give base materials for perfumes (2). In keeping with our current experience in antimicrobial testing (3) and bioautography (4) as selection guidelines during isolation procedures of marine natural products, crude extracts of this lichen were tested against *Bacillus subtilis*, *Escherichia coli*, *Penicillium digitatum*, and *Saccharomyces cerevisiae*, each of them being representative of a different class of microorganisms (gram-positive bacteria, gram-negative bacteria, fungi, and yeasts, respectively).

All the extracts exhibited strong activity against the former two and a modest activity against the fungus. Hence, by preparative tlc, besides the common cortical depsides atranorin and chloroatranorin (inactive), the active methyl β -orcinolcarboxylate has been isolated in good yield, for the first time from this species. Remarkably, its most pronounced activity is the antifungal one.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Spectra were recorded with the following instruments: ^1H and ^{13}C nmr, Varian XL 200, and Bruker WP 80; ms, LKB-Shimadzu 9000 S. The INEPT pulse sequence has been performed with the XL data system for the ^{13}C -nmr spectra. Analytical tlc was performed

on silica gel 60 F₂₅₄ plates (thickness 0.25 mm) from E. Merck. Detection of compounds was made by spraying with a solution of 1% ceric sulphate in 2N H₂SO₄ and subsequent heating at 100° for 10 min; thus, different compounds give differently colored spots. Details of the agar plate disc diffusion antimicrobial procedure are given elsewhere (3), as well as the bioautographic procedure (4).

PLANT MATERIAL.—*P. furfuracea* was collected on the cortex of old north-facing *Pinus pinea*, Lake Ampollino, Calabria, Italy. Voucher specimens are deposited at University of Catania.

EXTRACTION, ISOLATION, AND IDENTIFICATION OF ATRANORIN, CHLOROATRANORIN, AND METHYL β-ORCINOLCARBOXYLATE.—The dried and ground lichen thalli (15 g) were extracted (Soxhlet) with C₆H₆ (12 h). Room-temperature rotary evaporation of the C₆H₆ extract gave a gray solid. Silica gel tlc, using as solvent system CHCl₃-EtOAc with 2% HOAc (9:1) indicated that this solid consisted of several compounds; bioautographic detection with *E. coli* and *B. subtilis* showed that the spot at Rf 0.46 was strongly active as well as an unresolved band at Rf 0 to 0.17. Thus, ten 20×20 cm silica gel 60 F₂₅₄ plates (thickness 0.5 mm, Merck) were loaded each with 60 mg of the C₆H₆ extract. This preparative tlc afforded the isolation of atranorin (130 mg) and 5-chloroatranorin (170 mg), both inactive, and of the active compound at Rf 0.46. Further elution with CHCl₃-C₆H₆ (8:2) through three Sep-Pak silica cartridges, each of them loaded with 60 mg, and then through a small silica gel S column, 70-230 mesh, yielded pure methyl β-orcinolcarboxylate (95 mg; 0.63%). This compound was observed also, by tlc and ms, in a CHCl₃ extract obtained by room-temperature percolation of the solvent through the freshly collected lichen; thus, its presence is not a thermal artifact formed during work-up (5).

All three compounds were identified by comparison of their physical (mp) and spectral (¹H and ¹³C nmr and ms) properties with those reported in the literature (6-8).

Atranorin.—¹³C nmr (50.25 MHz, CDCl₃) ppm: 103.1 (C-1), 169.4 (C-2), 109.9 (C-3), 167.8 (C-4), 113.2 (C-5), 152.8 (C-6), 170.0 (C-7), 25.9 (C-8), 194.2 (C-9), 117.1 (C-1'), 163.2 (C-2'), 110.6 (C-3'), 152.3 (C-4'), 116.3 (C-5'), 140.2 (C-6'), 172.5 (C-7'), 24.3 (C-8'), 9.8 (C-9'), 52.7 (COOCH₃).

5-Chloroatranorin.—¹³C nmr (50.25 MHz, CDCl₃) ppm: 108.6 (C-1), 166.2 (C-2), 110.2 (C-3), 163.4 (C-4), 115.7 (C-5), 151.8 (C-6), 169.2 (C-7), 21.1 (C-8), 193.6 (C-9), 116.7 (C-1'), 162.9 (C-2'), 110.3 (C-3'), 149.0 (C-4'), 115.6 (C-5'), 139.9 (C-6'), 172.1 (C-7'), 24.0 (C-8'), 9.4 (C-9'), 52.3 (COOCH₃).

Methyl β-orcinolcarboxylate.—¹³C nmr (20.1 MHz, CDCl₃) ppm: 105.8 (C-1), 163.6 (C-2), 108.8 (C-3), 158.5 (C-4), 110.8 (C-5), 140.5 (C-6), 173.0 (C-7), 24.1 (C-8), 7.7 (C-9), 51.9 (COOCH₃).

The chemical shifts for these compounds agree well with those reported by Huneck (8), but some values for atranorin are in contrast with others previously reported (9).

Antimicrobial activity of methyl β-orcinolcarboxylate (μg applied), mm zone of inhibition: against *P. digitatum* (from Institute of Plant Pathology) (40), 29; filipin as control (24), 29; against *S. cerevisiae* (baker yeast), (400), 18; filipin as control (24), 21; against *B. subtilis* (ATCC 6633) (400), 17; streptomycin sulfate as control (0.6), 16; against *E. coli* (Strain B, ATCC 11303) (400), 15; streptomycin sulfate as control (6), 17.

From these data, the strongly antifungal methyl β-orcinolcarboxylate is mainly responsible for the modest antifungal activity of the extracts, while more polar compounds are responsible for the strong antibacterial activity of the extracts, as suggested by bioautography.

ACKNOWLEDGMENTS

We thank Dr. M. Valcuvia Passadore, Botany Institute, University of Pavia, for botanical identification. This study was supported by a grant from the Ministry of Public Education.

LITERATURE CITED

1. K.D. Vartia, "Antibiotics in Lichens," in: "The Lichens," Ed. by V. Ahmadjian and M.E. Hale, New York: Academic Press, 1973.
2. K. Bergwein, *Dragoco Rept.*, **11**, 110 (1964).
3. S. Caccamese and R. Azzolina, *Planta Med.*, **37**, 333 (1979).
4. S. Caccamese and R.M. Toscano, unpublished results.
5. C.F. Culberson, W.F. Culberson, and A. Johnson, *Phytochemistry*, **16**, 127 (1977).
6. S. Huneck, "Lichen Substances," in: "Progress in Phytochemistry" vol. 1, Ed. by L. Reinhold and Y. Liwischitz, New York, Wiley Interscience, 1968.
7. J. Santesson, *Ark. Kemi*, **30**, 363 (1969).
8. E.G. Sundholm and S. Huneck, *Chemica Scripta*, **16**, 233 (1980).
9. G. Nicollier and R. Tabacchi, *Helv. Chim. Acta*, **59**, 2979 (1976).