Pharmacol., **17**, 282 (1970).
(13) B. S. Draser, A. G. Renwick, and R. T. Williams, *Biochem. J.*, **129**, 881 (1972).
(14) H. Ichibagase, S. Kojima, K. Inoue, and A. Suenaga, *Chem. Pharm. Bull.*, **20**, 175 (1972).
(15) *Ibid.*, **20**, 1093 (1972).
(16) A. Suenaga, S. Kojima, and H. Ichibagase, *Chem. Pharm. Bull.*, **20**, 1357 (1972).
(17) T. Nimura, T. Tokieda, and T. Yamaha, *J. Biochem. (Tokyo)*, **75**, 407 (1974).
(18) J. J. Roxon and A. A. Tesorio, *Aust. J. Pharm. Sci.*, **NS3**, 26 (1974).
(19) J. J. Roxon and A. A. Tesorio, *Xenobiotica*, **5**, 25 (1975).
(20) G. A. Benson and W. J. Spillane, *J. Pharm. Sci.*, **65**, 1841 (1976).
(21) F. F. Blick, H. E. Millson, Jr., and N. J. Doorenbos, *J. Am. Chem. Soc.*, **76**, 2498 (1954).
(22) B. Unterhalt and L. Böschemeyer, *Z. Lebensm.-Unters.-Forsch.*, **145**, 93 (1971).
(23) W. J. Spillane and G. A. Benson, *J. Med. Chem.*, **19**, 869 (1976).
(24) L. F. Audrieth and M. Sveda, *J. Org. Chem.*, **9**, 89 (1944).
(25) P. H. Derse and R. J. Daun, *J. Assoc. Offic. Anal. Chem.*, **49**, 1090 (1966).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 26, 1976, from the *Department of Chemistry, University College, Galway, Ireland.*
Accepted for publication August 3, 1976.
The authors thank the Department of Education (Ireland) for a grant to G. A. Benson.
[x] To whom inquiries should be directed.

Cytotoxic Agents from *Michelia champaca* and *Talauma ovata*: Parthenolide and Costunolide

JOSEPH J. HOFFMANN, STERLING J. TORRANCE, RICHARD M. WIEDHOPF, and JACK R. COLE [x]

Abstract □ The ethanol extract of *Michelia champaca* and the petroleum ether extract of *Talauma ovata* showed activity toward the human epidermoid carcinoma of the nasopharynx test system. The active constituents were sesquiterpene lactones, identified as parthenolide ($C_{15}H_{20}O_3$) and costunolide ($C_{15}H_{20}O_2$). Their identities were proven by elemental analyses, PMR, IR, and mass spectral data, melting-point and mixed melting-point determinations, and comparisons with authentic samples and spectra.

Keyphrases □ Parthenolide—isolated from ethanol extract of bark of *Michelia champaca*, cytotoxic activity evaluated □ Costunolide—isolated from ethanol extract of bark of *Michelia champaca* and petroleum ether extract of roots of *Talauma ovata*, cytotoxic activity evaluated □ *Michelia champaca*—parthenolide and costunolide isolated from ethanol extract of bark, cytotoxic activity evaluated □ *Talauma ovata*—costunolide isolated from petroleum ether extract of roots, cytotoxic activity evaluated □ Cytotoxic activity—plant constituents parthenolide and costunolide evaluated

As a result of the continuing search for plants having tumor inhibitory constituents, it was found that the ethanol extract of the stem bark of *Michelia champaca* L.[1] (Magnoliaceae) and the petroleum ether extract of the roots of *Talauma ovata* A. St. Hil.[2] (Magnoliaceae) showed cytotoxic activity toward the human epidermoid carcinoma of the nasopharynx test system[3] (KB).

DISCUSSION

The cytotoxic agents were found to be parthenolide (from *M. champaca*) and costunolide (from *M. champaca* and *T. ovata*). Parthenolide previously was isolated from several Magnoliaceae plants, including *Magnolia grandiflora* (1) and *M. champaca* (2), and also from *Chrysanthemum parthenium* (Asteraceae) (3). Costunolide has been found mainly in Asteraceae plants, *e.g.*, the "costus root," *Saussurea lappa* (4). Identification of parthenolide and costunolide was achieved by IR, PMR, and mass spectral data and by comparison with authentic spectra and samples.

Parthenolide demonstrated an activity of 2.3 μg/ml, and costunolide showed an activity of 2.8 μg/ml. Activity in the KB test system is defined as $ED_{50} \leq 20$ μg/ml (5).

EXPERIMENTAL[4]

Isolation Procedure—The ground stem of *M. champaca* (7 kg) was extracted exhaustively with ethanol in a Lloyd-type extractor. The air-dried residue was partitioned between chloroform and water. The air-dried chloroform phase (11.8 g) was extracted twice with benzene (500 ml), and the benzene-soluble fraction (8 g) was subjected to silica gel 60 (200 g) column (4 × 46 cm) chromatography. The column was eluted with benzene (1000 ml), chloroform (1000 ml), chloroform–methanol [(95:5) six fractions, 500 ml each], acetone, and methanol.

The dried and ground roots of *T. ovata* (1.0 kg) were extracted by maceration with 3 liters of petroleum ether (bp 30–60°) overnight. After filtration and removal of the solvent from the extract, crude crystals of costunolide formed.

Isolation of Parthenolide—Parthenolide was isolated from the first chloroform–methanol (95:5) fraction by preparative TLC using benzene–dichloromethane–ethyl acetate (3:6:1). Crystallization from ether resulted in colorless plates, mp 115° [lit. (2) mp 115°]. The PMR spectrum was identical to the previously reported spectrum (2). The IR

[1] The plant was collected in India in March 1974. Identification was confirmed by Dr. Robert E. Perdue, Jr., Medicinal Plant Resources Laboratory, U.S. Department of Agriculture, Beltsville, Md. A reference specimen was deposited in that herbarium.
[2] The plant was collected in Brazil in June 1974. Identification was confirmed by Dr. Robert E. Perdue, Jr., Medicinal Plant Resources Laboratory, U.S. Department of Agriculture, Beltsville, Md. A reference specimen was deposited in that herbarium.
[3] Of the Drug Evaluation Branch, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare, Bethesda, Md.
[4] Carbon and hydrogen analyses were performed by Chemalytics, Inc., Tempe, Ariz. PMR, IR, and mass spectra were determined using a Varian T-60 spectrophotometer, a Beckman IR-33, and a Hitachi Perkin-Elmer double-focusing spectrometer (model RMU-6E), respectively. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

spectrum was superimposable with the IR spectrum of an authentic sample. The mixed melting point obtained showed no depression.

Anal.—Calc. for $C_{15}H_{20}O_3$: C, 72.17; H, 8.06; mol. wt. 248. Found: C, 72.38; H, 8.26; *m/e* 248.

Isolation of Costunolide—Costunolide was isolated from the chloroform fraction of *M. champaca* by crystallization from hexane–benzene, and the crude crystals from *T. ovata* were purified by recrystallization from hexane–benzene to give 352 mg of colorless spears, mp 106–107° [lit. (4) mp 106–107°]. The IR and PMR spectra were identical to the authentic spectra.

Anal.—Calc. for $C_{15}H_{20}O_2$: C, 77.54; H, 8.67; mol. wt. 232. Found: C, 77.32; H, 8.84; *m/e* 232.

REFERENCES

(1) R. M. Wiedhopf, M. Young, E. Bianchi, and J. R. Cole, *J. Pharm. Sci.*, **62**, 345 (1973).

(2) T. R. Govindachari, B. S. Joshi, and V. N. Kamat, *Tetrahedron*, **21**, 1509 (1965).

(3) V. Herout and F. Sŏrm, *Chem. Ind.*, **1959**, 1067.

(4) S. C. Bhattacharyya, G. R. Kelkar, and A. S. Rao, *ibid.*, **1959**, 1069.

(5) R. I. Geran, N. H. Greenberg, M. N. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep.*, **3** (3), 17 (1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received April 2, 1976, from the *Division of Pharmaceutical Chemistry, College of Pharmacy, University of Arizona, Tucson, AZ 85721.*

Accepted for publication July 28, 1976.

Supported by Contract N0I-CM-33750 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare, Bethesda, MD 20014, and the Elsa U. Pardee Foundation, Midland, MI 48640.

The authors are indebted to Dr. B. S. Joshi, Ciba of India Ltd., Goregaon Bombay, India, for providing the authentic sample of parthenolide, and to Professor Raymond W. Doskotch, Ohio State University, Columbus, Ohio, for providing authentic spectra (IR and NMR) of costunolide. A specimen was unavailable because of prior decomposition.

[x] To whom inquiries should be directed.

NMR Solvent Shift Data for Methoxylated Xanthones

R. A. FINNEGAN [x] and K. E. MERKEL

Abstract □ The NMR spectra of 1-, 2-, 3-, and 4-hydroxyxanthones, 1,3-, 1,5-, 1,6-, 1,7-, 1,8-, 2,5-, 3,4-, 3,5-, 3,6-, and 4,5-dihydroxyxanthones, 1,3,6- and 1,3,8-trihydroxyxanthones, and 1,3,6,8-tetrahydroxyxanthone, as well as those of the corresponding methyl ethers and acetates, were recorded. The spectra of the methyl ethers were measured in deuterochloroform, benzene, trifluoroacetic acid, and 3% trifluoroacetic acid in benzene. The solvent shift parameters for the methoxyl resonances are tabulated and discussed.

Keyphrases □ Methoxyxanthones, various—NMR spectra and solvent shift data □ NMR—spectra and solvent shift data, various methoxyxanthones □ Xanthones, methoxy substituted—NMR spectra and solvent shift data

Recently, the isolation of four simple hydroxylated xanthones from seed extracts of *Mammea americana* L. was reported (1). They were identified as the 2- and 4-monohydroxy derivatives and the 1,7- and 1,5-dihydroxy derivatives by a combination of spectroscopic studies and syntheses of authentic materials (1). The last substance, in fact, was prepared for the first time. The 2,5- and 4,5-dihydroxy isomers were also prepared (2). During this work, other hydroxylated xanthones (3, 4) were prepared, not only for comparison purposes but also for inclusion in a preliminary antitumor screen (5).

The chemical shifts of methoxy resonances in some methoxyxanthone derivatives were dependent on the position of substitution (3). The magnitude of the benzene-induced solvent shift for the methyl group in substituted anisoles was dependent on the nature and orientation of the substituents (6). These facts suggested that benzene-induced shifts of methoxyl resonances in methoxyxanthones also might be position dependent and, therefore, might be helpful in elucidating the structures of naturally occurring hydroxyxanthones and methoxyxanthones.

While this work was in progress, benzene-induced shifts of methoxyl resonances in flavonoids (7–11) and substituted xanthones (12, 13) were reported and, indeed, found to be of use in structure determination. Useful solvent shift correlations were previously derived for steroidal ketones (14, 15) and substituted coumarins (16). In addition, trifluoroacetic acid (I) was found to be a useful adjunct to the

Table I—Chemical Shifts (Parts per Million) for Methoxyl Resonances of Various Methoxyxanthones in Various Solvents

Xanthone	Trifluoroacetic Acid	Deuterochloroform	Benzene	Benzene–Trifluoroacetic Acid
1-Methoxy	4.50	4.05	3.43	3.39
2-Methoxy	4.12	3.94	3.29	3.43
3-Methoxy	4.18	3.95	3.18	3.16
4-Methoxy	4.27	4.05	3.33	3.32
1,8-Dimethoxy	4.37	3.99	3.42	3.40
3,6-Dimethoxy	4.18	3.95	3.19	3.20
4,5-Dimethoxy	4.22	4.08	3.34	3.30
1,3-Dimethoxy	4.42	4.00	3.38	3.37
	4.23	3.92	3.22	3.22
1,5-Dimethoxy	4.50	4.03	3.41	3.36
	4.28	4.03	3.34	3.30
1,6-Dimethoxy	4.44	4.03	3.45	3.45
	4.20	3.92	3.16	3.15
1,7-Dimethoxy	4.48	4.04	3.44	3.41
	4.13	3.92	3.27	3.33
2,5-Dimethoxy	4.27	4.05	3.38	3.40
	4.12	3.95	3.29	3.33
3,4-Dimethoxy	4.30	4.05	3.84	3.75
	4.22	4.03	3.25	3.22
3,5-Dimethoxy	4.29	4.06	3.38	3.35
	4.17	3.94	3.11	3.12
1,3,6-Trimethoxy	4.38	3.99	3.44	3.42
	4.18	3.91	3.28	3.33
	4.18	3.91	3.22	3.25
1,3,8-Trimethoxy	4.34	3.98	3.43	3.45
	4.25	3.95	3.37	3.38
	4.17	3.89	3.22	3.26
1,3,6,8-Tetramethoxy	4.26	3.94	3.42	3.38
	4.15	3.88	3.26	3.38