synthesis of II. However 50% aqueous methanol is preferred for the analysis of pure II, since it yields a linear calibration line with a higher correlation coefficient.

REFERENCES

(1) M. J. Weinstein, G. M. Luedemann, E. Oden, and G. H. Wagman, *Antimicrob. Agents Chemother.*, **1964**, 24.

(2) G. H. Wagman, G. M. Luedemann, and M. J. Weinstein, *ibid.*, **1964**, 33.

(3) H. L. Herzog, E. Meseck, S. DeLorenzo, A. Murawski, W. Charney, and J. P. Rosselet, *Appl. Microbiol.*, **13**, 515 (1965).

(4) A. K. Ganguly, O. Z. Sarre, D. Greeves, and J. Morton, *J. Am. Chem. Soc.*, **97**, 1982 (1975).

(5) A. K. Ganguly, S. Szmulewicz, O. Z. Sarre, and V. M. Girijavallabhan, *J. Chem. Soc., Chem. Commun.*, **1976**, 609.

(6) P. Kabasakalian, S. Kalliney, A. K. Ganguly, and A. Westcott, U.S. pat. 3,998,708 (1976).

(7) P. E. Iversen and H. Lund, *Anal. Chem.*, **41**, 1322 (1969).

(8) L. C. Schroeter, *J. Pharm. Sci.*, **52**, 559 (1963).

Chemical Constituents of Gentianaceae XXIV: Anti-*Mycobacterium tuberculosis* Activity of Naturally Occurring Xanthones and Synthetic Analogs

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Received May 17, 1977, from the *Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Banaras Hindu University, Varanasi-5, India.* Accepted for publication August 24, 1977.

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Abstract □ Anti-*Mycobacterium tuberculosis* H37 RV data are presented for the individual xanthones of *Canscora decussata* Schult and *Swertia purpurascens* Wall (Gentianaceae); a few, from the former species, showed significant activity. Additionally, structure–activity relationships of these compounds are evaluated on the basis of the minimum inhibitory concentration data of 18 naturally occurring xanthones bearing 1,3,5-, 1,3,5,6-, 1,3,6,7-, 1,3,5,8-, 1,3,5,6,7-, and 1,3,6,7,8-oxygenated patterns and six synthetic analogs.

Keyphrases □ Xanthones, various—naturally occurring and synthetic, anti-*Mycobacterium tuberculosis* activity evaluated *in vitro* □ *Mycobacterium tuberculosis*—effect of various naturally occurring and synthetic xanthones *in vitro* □ Structure–activity relationships—various naturally occurring and synthetic xanthones evaluated for anti-*Mycobacterium tuberculosis* activity *in vitro*

A previous paper (1) described the significant anti-*Mycobacterium tuberculosis* activity of the total polyoxygenated xanthones of *Canscora decussata* Schult (Gentianaceae). This paper reports the identification of the potent anti-*M. tuberculosis* components of this plant. Additionally, the microbiological screening of synthetic analogs was conducted to evaluate the importance of the number and patterns of oxygenation of xanthones for this type of biological activity.

EXPERIMENTAL

Compounds—Xanthones I–IX, XII–XV, and XX–XXIV were available from previous investigations of *C. decussata* and *Swertia* species. Xanthone X was obtained by methylating IX with ethereal diazomethane. On permethylation with dimethyl sulfate and potassium carbonate in acetone under reflux (46 hr), IX gave XI. Xanthones XVI–XIX were prepared by using previously reported procedures (Table I).

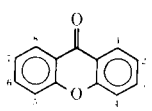
References to the isolation or preparation of the compounds are given in the table.

Test—A description of the screening method of *M. tuberculosis* H37 RV growth inhibitor activity was given previously (1). The minimum inhibitory concentration (MIC) of the test compounds required for preventing the microorganism growth was recorded (Table I). The compounds that could not prevent growth of the microorganism up to a dose of 200 μg/ml were considered inactive.

RESULTS AND DISCUSSION

The MIC data (Table I) of the 24 xanthones suggest that, for a moderate to significant anti-*M. tuberculosis* activity, the xanthone nucleus should contain oxygen functions at 1,3- and 5,6- or 8-positions. Among the seven types of oxygenated xanthones tested for this activity, 1,3,5,6,7- and 1,3,6,7,8-pentaoxygenated xanthones (XII and XVIII, respectively) were the most potent. Furthermore, in these two types of oxygenated

Table I—Growth Inhibitor Activity of Polyoxygenated Xanthones against *M. tuberculosis* H37 RV



Number	Xanthone	Reference	MIC, mcg/ml
I	1,3,5-Trihydroxy	2	200
II	1-Methoxy-3,5-dihydroxy	2	Inactive
III	1,5-Dihydroxy-3-methoxy	3	Inactive
IV	1-Glucosyloxy-3-hydroxy-5-methoxy	4	Inactive
V	1-Hydroxy-3,5-dimethoxy	3	Inactive
VI	1,3,5,6-Tetrahydroxy	5	10
VII	1,3,5-Trihydroxy-6-methoxy	3	40
VIII	1,6-Dihydroxy-3,5-dimethoxy	6	100
IX	1,3,6,7-Tetrahydroxy	5	40
X	1-Hydroxy-3,6,7-trimethoxy	5	100
XI	1,3,6,7-Tetramethoxy	5	100
XII	1,3,6-Trihydroxy-5,7-dimethoxy	7	5
XIII	1,6,7-Trihydroxy-3,5-dimethoxy	7	40
XIV	1,5,6-Trihydroxy-3,7-dimethoxy	7	10
XV	7-Glucosyloxy-1,6-dihydroxy-3,5-dimethoxy	4	100
XVI	1,3,8-Trihydroxy	7, 8	10
XVII	1,8-Dihydroxy-3-methoxy	7, 8	40
XVIII	1,3,6,7,8-Pentahydroxy	7, 8	5
XIX	1,3,6-Trihydroxy-7,8-dimethoxy	7, 8	10
XX	1,3,5,8-Tetrahydroxy	9	100
XXI	1,5,8-Trihydroxy-3-methoxy	10	200
XXII	1,3,8-Trihydroxy-5-methoxy	10	200
XXIII	1-Glucosyloxy-3,5,8-trihydroxy	9	200
XXIV	1-Glucosyloxy-3-methoxy-5,8-dihydroxy	9	200
	Streptomycin sulfate[a]		0.5

[a] Standard antitubercular drug used for comparison of activity against this strain.

xanthones, those containing hydroxyl groups at 1,3- and 6- or 8-positions were more active than those containing other substituents (methoxy or glucosyloxy) at these positions.

Norswertianolin (1-glucosyloxy-3,5,8-trihydroxyxanthone) (XXIII) and swertianolin (1-glucosyloxy-3-methoxy-5,8-dihydroxyxanthone) (XXIV), which occur in a number of *Swertia* species (9–11), were previously reported to produce antitubercular activity, although their exact potency was not reported (11). In the present investigation, XXIII and XXIV exhibited only a weak anti-*M. tuberculosis* activity. The aglucone (XX) of XXIII appeared to be more active than the corresponding 1-*O*-glucosyl derivative, suggesting the importance of the free hydroxyl group at the 1-position for activity.

Hansch analysis (12) with the partition coefficients (π) and MIC values of I–XXIV was attempted but did not provide a good correlation. Electronic and steric factors also seemed to be responsible for the observed biological activity.

REFERENCES

(1) S. Ghosal and R. K. Chaudhuri, *J. Pharm. Sci.*, **64**, 888 (1975).

(2) S. Ghosal, K. Biswas, R. B. P. S. Chauhan, and R. K. Chaudhuri, *Phytochemistry*, **15**, 1041 (1976).

(3) R. K. Chaudhuri and S. Ghosal, *ibid.*, **10**, 2425 (1971).

(4) S. Ghosal, K. Biswas, and R. K. Chaudhuri, *J. Chem. Soc. Perkin Trans.*, **1**, 1597 (1977).

(5) S. Ghosal and R. K. Chaudhuri, *Phytochemistry*, **12**, 2035 (1973).

(6) S. Ghosal, R. K. Chaudhuri, and A. Nath, *J. Pharm. Sci.*, **62**, 137 (1973).

(7) S. Ghosal, R. K. Chaudhuri, and K. R. Markham, *J. Chem. Soc. Perkin Trans.*, **1**, 2538 (1974).

(8) R. K. Chaudhuri, Ph.D. thesis, Banaras Hindu University, Varanasi, India, 1973, p. 127.

(9) S. Ghosal, P. V. Sharma, and R. K. Chaudhuri, *J. Pharm. Sci.*, **63**, 1286 (1974).

(10) S. Ghosal, P. V. Sharma, R. K. Chaudhuri, and S. K. Bhattacharya, *ibid.*, **62**, 926 (1973).

(11) M. Komatsu and T. Tomimori, Japanese pat. 7,216,676 (1972); through *Chem. Abstr.*, **77**, 8578 (1972).

(12) C. Hansch, in "Drug Design," vol. 1, E. J. Ariens, Ed., Academic, New York, N.Y., 1971, p. 271.

ACKNOWLEDGMENTS

The authors thank Dr. M. H. Shah, Haffkine Institute, Bombay, India, for providing the microbiological screening data and Prof. Dr. F. Zymalkowski, Pharmazeutisches Institut der Universität, Bonn, West Germany, for providing counsel and laboratory facilities to R. K. Chaudhuri. K. Biswas thanks the Council of Scientific and Industrial Research, New Delhi, India, for a Research Fellowship.

Characterization of Products Derived from Aprindine Hydrochloride Photolysis

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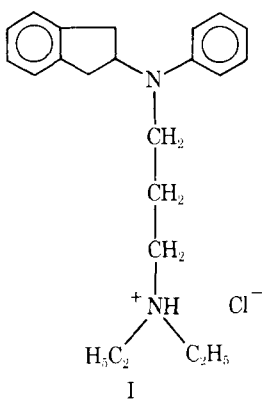
Received March 7, 1977, from the *Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46206.* Accepted for publication August 29, 1977.

Abstract □ An investigation of the products resulting from the photolysis of aprindine hydrochloride is described. The compounds were characterized by GLC spiking experiments and combined GLC–mass spectrometry. In some cases, R_f values derived from TLC and/or preparative TLC and subsequent high-resolution mass spectral measurements also were employed for identification.

Keyphrases □ Aprindine hydrochloride—photolysis products identified by GLC and combined GLC–mass spectrometry □ GLC and combined GLC–mass spectrometry—identification of photolysis products of aprindine hydrochloride □ Photolysis—aprindine hydrochloride, products identified by GLC and GLC–mass spectrometry □ Cardiac depressants—aprindine hydrochloride, photolysis products identified by GLC and GLC–mass spectrometry

In a previous report (1), a GLC method was presented which effectively monitors the stability of the antiarrhythmic agent aprindine hydrochloride (2) [*N,N*-diethyl-*N'*-(2-indanyl)-*N'*-phenyl-1,3-propanediamine hydrochloride] (I). During this work, it was discovered that I is not stable to UV irradiation, as evidenced by extra peaks in the gas chromatograms obtained from GLC analysis of the photolysis solutions. Tentative identifications were suggested on the basis of GLC retention times, but detailed characterizations were not reported.

This report describes the characterization of the aprindine photoproducts *via* TLC, GLC, and combined GLC–mass spectrometry.



I

EXPERIMENTAL

GLC and combined GLC–mass spectrometric measurements were obtained using the instruments previously described (1). For this work, however, the combined gas chromatograph–mass spectrometer was interfaced to a data system[1] that provided a background subtraction routine as an option. Additionally, nonlinear temperature programming provided optimal resolution.

A typical run was carried out as follows: the oven was equilibrated at 120°, the sample was injected, and the chromatogram was recorded isothermally for 3.0 min. The oven temperature was then raised to 190°.

[1] The 150 data system, Systems Industries, Sunnyvale, Calif.