Anal.—Calc. for $C_{11}H_{10}N_2O_2$: C, 65.33; H, 4.98; N, 13.86. Found: C, 65.41; H, 4.80; N, 13.90.

2-[2-(Piperidino)ethyl]-10,10a-dihydro -1*H*,5*H*- imidazo[1,5-*b*]isoquinoline-1,3(2*H*)-dione Hydrochloride (II)—A mixture of 26.5 g (0.131 mole) of III, 48.2 g (0.262 mole) of V, 54.2 g (0.393 mole) of potassium carbonate, and 19.7 g (0.131 mole) of sodium iodide in 900 ml of dimethyl sulfoxide was stirred at 50–55° for 48 hr. An additional 24.1 g (0.131 mole) of V was added to the mixture, and stirring was continued at 50–55° for an additional 24 hr. Then the mixture was cooled, poured into 2.0 liters of cold water, and extracted with 4 × 400 ml of chloroform. The combined extracts were washed with 2 × 800 ml of water, dried (magnesium sulfate), and concentrated to dryness. The oil was boiled with 180 ml of ethanol, and the resulting suspension was stored in the refrigerator for 2 weeks. Filtration gave 9.0 g of unreacted III.

To the filtrate was added 40 ml of methanol saturated with hydrogen chloride. The resulting solid was recrystallized from 125 ml of ethanol to yield, after drying at 100° for 4 hr, 7.9 g (26% based on consumed III) of the product, mp 204–207°. An analytical sample, mp 237–239°, was obtained by recrystallization from ethanol; IR: 5.70 and 5.90 (C=O, imidazolidinedione) μm; NMR (dimethyl sulfoxide-d_6): δ 1.46–1.96 (broad m, 6, piperidine 3-CH_2, 4-CH_2, and 5-CH_2), 3.05–3.20 (m, 2, C-10 H_2), 3.16–3.50 (broad m, 4, piperidine 2-CH_2 and 6-CH_2), 3.73–5.00 (m, 7, C-5 H_2, C-10a H, and NCH_2CH_2N), 7.28 (s, 4, aromatic CH), and 10.9–11.4 (broad s, 1, exchangeable NH) ppm.

Anal.—Calc. for $C_{18}H_{23}N_3O_2$·HCl: C, 61.79; H, 6.91; N, 12.01. Found: C, 61.78; H, 7.02; N, 11.86.

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dc Polarographic Determination of Hydroxylaminoeverninomicin D

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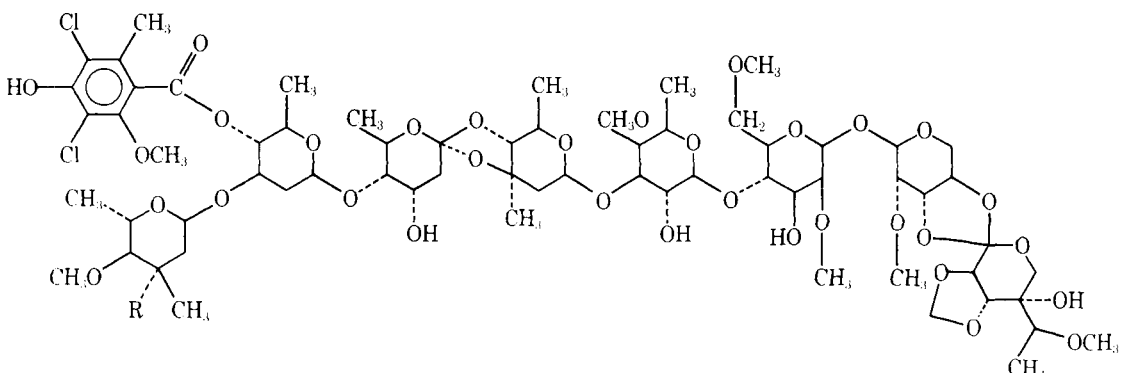
Received June 24, 1977, from the *Development Division, Schering Corporation, Bloomfield, NJ 07003.* Accepted for publication August 18, 1977.

Abstract □ An analytical procedure for a tertiary alkylhydroxylamine, hydroxylaminoeverninomicin D, was developed. It involved the autoxidation of the compound in the presence of Cu(II) and subsequent polarographic reduction. Conditions are described for the quantitative determination of the possible impurities (everninomicin D and nitrosoeverninomicin D) in hydroxylaminoeverninomicin D.

Keyphrases □ Hydroxylaminoeverninomicin D—polarographic analysis, bulk drug □ Polarography—analysis, hydroxylaminoeverninomicin D, bulk drug □ Antibacterials—hydroxylaminoeverninomicin D, polarographic analysis, bulk drug

Everninomicins (1–3) produced by *Micromonospora carbonaceae* are highly active oligosaccharide antibiotics against Gram-positive bacteria including strains resistant to penicillins, tetracyclines, lincomycins, rifampin, macrolides, and chloramphenicol. The major component of the everninomicin complex (4) is everninomicin D (I). The chemical (5) or electrochemical (6) reduction of the tertiary nitro group in I yields hydroxylaminoeverninomicin D (II). Nitrosoeverninomicin D (III), undergoing similar reductions, also yields II. Compounds I–III possess equal *in vitro* activity against Gram-positive bacteria. However, II gives the highest blood level when administered intramuscularly to dogs.

A reported polarographic determination of alkylhydroxylamines was based on the formation of reproducible



I: R = NO_2
II: R = NHOH
III: R = NO

Table I—Effect of pH on the Autoxidation of Hydroxylaminoeverninomicin D[a] in the Absence and Presence of Cu(II) in 50% Aqueous Methanol

pH[b]	$-E_{1/2}$ *versus* NCE, v	Cu(II)[c]	Aeration Time, min	Diffusion Current, μamp
9	0.40	Absent	15	0.20
		Absent	30	0.22
		Present	15	1.10
		Present	30	1.10
	1.24	Absent	15	0.52
		Absent	30	0.58
		Present	15	0.14
		Present	30	0.11
10	0.43	Absent	15	0.23
		Absent	30	0.25
		Present	15	1.15
		Present	30	1.17
	1.24	Absent	15	0.55
		Absent	30	0.65
		Present	15	0.00
		Present	30	0.00
11	0.47	Absent	15	0.24
		Absent	30	0.30
		Present	15	1.09
		Present	30	1.11
	1.23	Absent	15	0.56
		Absent	30	0.74
		Present	15	0.15
		Present	30	0.13
12	0.52	Absent	15	0.72
		Absent	30	0.85
		Present	15	0.94
		Present	30	0.93
	1.26	Absent	15	1.58
		Absent	30	1.98
		Present	15	0.11
		Present	30	0.13

[a] Concentration = 1 mg/ml. [b] Tertiary amine organic buffers. [c] Cu(II) = 10 ppm.

anodic waves in alkaline media (7). Since, in the presence of oxygen, alkylhydroxylamines yield irreproducible anodic waves which cannot be removed by nitrogen flushing, oxygen was removed using an alkaline sulfite solution.

Unfortunately, this procedure failed for II, since sulfite oxidation for the removal of oxygen is inhibited (8) in the presence of organic solvents (acetonitrile, dimethylformamide, and methanol) in which everninomicins must be prepared because of their poor solubility in water.

A polarographic analytical procedure was developed to assay II during synthesis and as a finished product. This method is based on the production of polarographically reducible functional groups in the autoxidation of II catalyzed by Cu(II) ions.

EXPERIMENTAL

Apparatus—All polarograms were recorded on a polarograph[1] equipped with a 3-ml H-type cell containing a normal calomel electrode (NCE) and a dropping mercury electrode. The cell compartments were separated by an agar plug and a fritted-glass diaphragm. The chloride ions in both the calomel cell and the agar plug were furnished by tetramethylammonium chloride.

Chemicals—All chemicals were the highest quality commercially available. Separate standard solutions of I–III were prepared from pure powder[2] in acetonitrile, dimethylformamide, or methanol.

Procedure—Separate stock solutions (>2 mg/ml) of I–III were prepared in acetonitrile, dimethylformamide, or methanol. Five milliliters of triethylamine (0.05 *M*)–hydrochloric acid (0.007 *M*) buffer and a volume of the stock solution containing 10 mg of I, II, or III were put into a 10-ml volumetric flask. The mixture was diluted to volume with the corresponding organic solvent. An aliquot was transferred to the polarographic cell and deaerated with prewashed (with a blank solution) argon for about 15 min.

The applied potential range was from 0.00 to −1.50 v (normal calomel electrode), and the current range was set at 2 μamp. Fifty microliters of 7 mg of Cu(II)/ml in 2 *N* ammonium hydroxide was added to the 10-ml volumetric flask and allowed to stand exposed to air at room temperature for 15 min. A second polarogram was obtained using an aliquot of the Cu(II)-containing mixture. The first polarogram served as a blank for the second one.

The mercury flow rate at an open circuit with mercury dropping into 0.1 *M* potassium chloride was 1.16 mg/sec, the drop time was 5.48 sec, and the mercury column height was 61.4 cm.

[1] Leeds and Northrup Electro-Chemograph Type E.
[2] Schering Corp., Bloomfield, N.J.

Table II—Effect of Cu(II) Concentration on the Autoxidation of Hydroxylaminoeverninomicin D[a] in 50% Aqueous Methanol at pH 10

Cu(II) Concentration, ppm	$-E_{1/2}$ *versus* NCE, v	Aeration Time, min	Diffusion Current, μamp
0	0.43	0	0.00
		15	0.16
		30	0.26
	1.24	0	0.00
		15	0.52
		30	0.76
5	0.43	0	0.48
		15	1.06
		30	1.06
	1.25	0	0.32
		15	0.00
		30	0.00
10	0.46	0	1.30
		15	1.14
		30	1.12
	1.24	0	0.07
		15	0.00
		30	0.00
25	0.44	0	1.44
		15	1.44
		30	1.53
50	0.46	0	1.01
		15	1.05
		30	1.04
100	0.44	0	1.38
		15	1.05
		30	1.02

[a] Concentration = 1 mg/ml.

RESULTS AND DISCUSSION

Compound II was not reduced polarographically over the potential range between 0.0 and −2.0 v (normal calomel electrode). However, buffered basic solutions (tertiary amine organic buffers) in 50% aqueous methanol, on standing in air, developed two polarographic reduction waves (Table I). The magnitude of these reduction currents depended on pH and time.

The presence of Cu(II) had a catalytic effect, increasing the first reduction wave ($E_{1/2} \sim -0.4$ v) while reducing the second wave ($E_{1/2} \sim -1.2$ v). At pH 10, the second wave was completely eliminated. With this optimum pH of 10, the effect of Cu(II) concentration on the reduction waves was determined (Table II). Between 0 and 30 min of aeration, the least amount of diffusion current variation occurred at 50 ppm of Cu(II). Consequently, pH 10 and 15 min aeration time were chosen as the optimum conditions for constructing a linear standard curve for II over a 0.1–1.6-mg/ml range. The slope of the diffusion current (measured at −0.6 v) *versus* the concentration curve of II was 0.80 μamp/mg/ml, while the detection limit was about 0.05 mg/ml.

Although I and III are not autoxidizable even in the presence of Cu(II), they yield polarographic reduction waves having nearly identical half-wave potentials ($E_{1/2}$) of about −1.2 v under the described conditions. When either one or both of these compounds are present as impurities in II, the total content of I and III can be determined from the first polarogram run in the absence of Cu(II).

Fifty percent aqueous acetonitrile or dimethylformamide solution can be used successfully in this procedure for following the electrochemical

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.

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

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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).

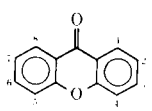
References to the isolation or preparation of the compounds are given in the table.

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.