The resulting pale yellow solution was poured into 600 ml. of ice and water and XIV precipitated out (yield 95%). It was dried and sublimed at 3 mm. pressure. White crystals of XIV were obtained which melted at 104–105.5°. The infrared spectrum exhibited the expected strong secondary amide linkage at 1665 cm.$^{-1}$, strong acetate absorption at 1220 cm.$^{-1}$, and C–Cl absorption from 700–800 cm.$^{-1}$.

Anal. Calcd. for $C_{14}H_{11}Cl_2NO$: Cl, 25.32. Found: Cl, 25.19.

Biological Results.—Screening of the compounds prepared was done with 2-month old Swiss Albino mice.[26] The compounds listed in Table I were screened in the Biology Department of Hunter College.[27] The procedure used was the established technique of the Sloan–Kettering Institute,[28] except that instead of injecting 24 hr. after implantation of tumors, injections began between 72 and 96 hr. after implantation. The vehicle used was Planters Peanut Oil. The mice were sacrificed the eighth day after implantation. Carcinostatic activity was obtained with II, VIII, XII, and a combination of II and XII at the specified dosages. Since we have had regression with 2-fluoro-9,10-phenanthraquinone (XV),[25] it was of interest to try a combination of XV, XII, and II. In a test using VIII at 125 mg./kg./day, the tumor on one of the test mice, which was definitely measurable, completely regressed by the eighth day. Dr. H. Christine Reilly of Sloan-Kettering Institute has obtained a tumor diameter measurement of T/C in cm. of 75% for a testing of XII at 250 mg./kg./day and 92% for V at 50 mg./kg./day.[29]

Acknowledgment. The authors wish to thank Dr. H. Christine Reilly and Dr. C. Chester Stock for helpful discussions and communications as well as Phillip Pfeffer and Steven Rosenberg for their technical assistance.

(26) Mice, (18–20 g.) were obtained from Davies and Nagle Inc., New York, N. Y.

(27) The Sarcoma 180 was obtained from Dr. H. Christine Reilly. The screenings were assisted by Steven Rosenberg, Henry Clayman, and Virginia Wevurski.

(28) *Cancer Res.*, Suppl. No. 1, 91 (1953); Suppl. No. 2, 179 (1955); *Cancer Res.*, **18**, No. 8, 49 (1958).

(29) Private communication from Dr. H. Christine Reilly.

The Antileukemia Activity of Tylocrebrine

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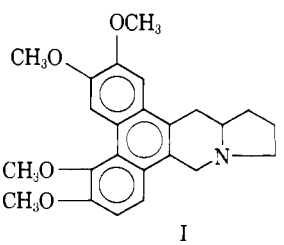
Received November 16, 1963

The isolation, structure determination, and synthesis of the alkaloid, tylocrebrine, from the North Queensland slender vine, *Tylophora crebriflora*, has been reported previously.[1] This levorotatory alkaloid was shown to have a phenanthroindolizidine structure (I).



I

Tylocrebrine has been tested against three tumor systems by the Cancer Chemotherapy National Service Center, U. S. Department of Health, Education, and Welfare, Bethesda, Md., in accordance with the protocols[2a] governing their screening program. The following is a report of the results obtained.

(1) E. Gellert, T. R. Govindachari, M. V. Lakshmikantham, I. S. Ragade, R. Rudzats, and N. Viswanathan, *J. Chem. Soc.*, 1008 (1962).

TABLE I

SARCOMA 180

Dose, mg./kg.	Survivors, X out of Y		Average tumor weight in mg. Test	Control	%, test/control
12.0	4	6	1185	1881	62

TABLE II

ADENOCARCINOMA 755

Dose, mg./kg.	Survivors, X out of Y		Average tumor weight in mg. Test	Control	%, test/control
10.0	9	10	735	1138	64

Methods

Test animals used as hosts were noninbred albino mice (Swiss) in the case of Sarcoma 180,[2b] and BDF_1 hybrid mice in the cases of Adenocarcinoma 755[2c] and Lymphoid Leukemia L1210.[2d]

All injections were administered intraperitoneally once a day except in the case of Sarcoma 180 when two injections per day were given. The solvent employed as vehicle of drug administration was carboxymethylcellulose with the exception of one series of tests involving Lymphoid Leukemia L1210, when another solvent (two drops of Tween 80 diluted to 20 ml. with saline) was used.

Mice were sacrificed after fourteen doses, *i.e.*, on the eighth day of administration in the case of Sarcoma 180; after eleven doses, *i.e.*, on the twelfth day in the case of Adenocarcinoma 755; while in the case of Lymphoid Leukemia L1210, administration of the drug was continued until death.

Results

Inactive Tests.—Against either Sarcoma 180 or Adenocarcinoma 755 administration of tylocrebrine at nontoxic dose levels showed insufficient decrease in the tumor weight of mice in comparison with the control animals.

Active Tests.—Administration of tylocrebrine to the Lymphoid Leukemia L1210 system considerably increased the survival time of the test animals. There are variations in the increases observed which appear to depend mainly upon the size of the administered dose, but occasional variations within the same dose range were also observed. The criterion of reproduc-

TABLE III

LYMPHOID LEUKEMIA L1210

Dose, mg./kg.	Survivors for at least 6 days, X out of Y		Average survival time of animals in days Test	Control	%, test/control
15.0	5	6	11.0	9.1	120
	6	6	15.0	9.9	151
10.0	6	6	14.0	9.9	141
	6	6	13.7	8.7	157
	6	6	14.0	8.3	168
	6	6	13.8	9.1	151
6.6	6	6	11.6	9.9	117
	6	6	11.7	9.1	128
4.4	6	6	12.5	9.9	126
	6	6	11.7	9.1	128
2.2	6	6	10.6	9.9	107

(2) (a) Protocols for Screening Chemical Agents and Natural Products against Animal Tumors and other Biological Systems, in *Cancer Chemotherapy Rept.*, No. 25 (1962); (b) Protocol 1.301; (c) Protocol 1.302; (d) Protocol 1.303; (e) Protocol 12.303.

ible activity in this tumor is a $T/C \leq 150\%$ at the optimal dose.[2e]

Conclusions

Tylocrebrine shows high activity against Lymphoid Leukemia L1210 in mice, and these tests indicate that best results are likely to be obtained at a dose level of about 10 mg./kg. The activity ascertained in these tests is sufficient for scheduling this compound for preclinical pharmacology and, in the absence of prohibitive toxicity, for large-scale clinical testing.

Acknowledgment.—The authors wish to express thanks to Dr. J. L. Hartwell, Chief of the Research Communications Branch, Cancer Chemotherapy National Service Center, National Cancer Institute, Bethesda 14, Md., for his interest in this project.

1,2-Disubstituted Naphth[2,3-*d*]imidazole-4,9-diones and Corresponding Quaternary Salts[1]

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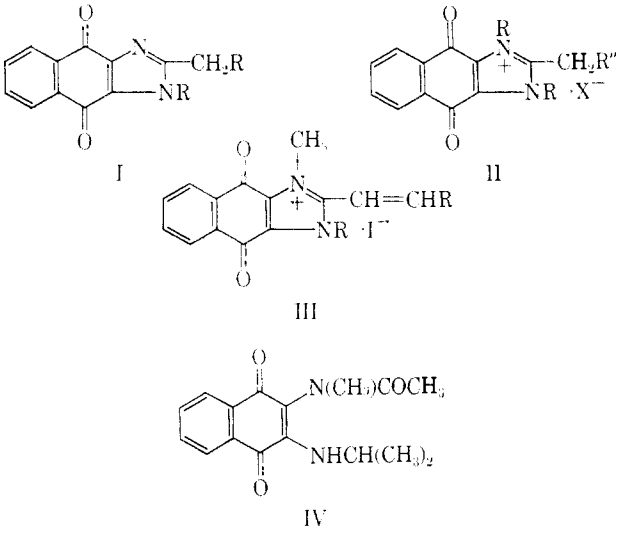
Received August 5, 1963

Hoover and Day[2] described the preparation and properties of a number of 2-alkyl-1H-naphth[2,3-*d*]-imidazole-4,9-diones. Some of these compounds were reported to have inhibitory activity against *Escherichia coli* 113-3 and B96.[2] The present work deals with the preparation of 1-alkyl- and 1-aryl-2-methylnaphth-[2,3-*d*]imidazole-4,9-diones (I), quaternary salts of these compounds, and a study of various properties of these substances.

2-Acetamido-3-alkyl- or -3-aryl-1,4-naphthoquinones were prepared according to the directions of Truitt, *et al.*[3] These compounds were converted to the imidazoles (I) by the action of 2 *N* sodium hydroxide as directed by Fries and Billig[4] and utilized by Hoover and Day.[2] It is interesting to note in our work that the expected 2-acetamido-3-anilino-1,4-naphthoquinone was obtained if an ethanol solution of aniline and 2-acetamido-3-chloro-1,4-naphthoquinone (2:1 mole ratio) was refluxed. However, if the reactants were used in a 1:1 mole ratio, 2-methyl-1-phenylnaphth-[2,3-*d*]imidazole-4,9-dione was produced in good yield. *p*-Bromoaniline and *p*-toluidine gave similar results but with lower yields. Alkyl amines did not give imidazoles under similar condition.

When the imidazoles (I) were heated with methyl iodide (or other reactive halides) the quaternary salts (II) were obtained. These compounds melted with vigorous evolution of a gas. The pyrolysis of II (R



I II III IV

= isopropyl, R′ = methyl, R″ = H, and X = I) gave isopropyl iodide and methyl iodide in a 10:1 ratio, as determined by gas chromatography.

Although Hoover and Day[2] reported that 1H-2-methylnaphth[2,3-*d*]imidazole-4,9-dione would not react with aldehydes in the presence of bases, we found that the quaternary salts (II) gave the expected styryl derivatives (III) when refluxed with benzaldehyde in the presence of piperidine or pyrrolidine.

Strong bases, such as NaOH, opened the imidazolium ring. For example, 4,9-dihydro-4,9-dioxo-1-dimethyl-3-(2-propyl)naphth[2,3-*d*]imidazolium iodide reacted with cold sodium hydroxide to give only 2-(*N*-methyl-acetanido)-3-(2-propylamino)-1,4-naphthoquinone(IV)

Physiological Acitivity.[5]—Compounds **15** and **16** (Table II) were not more than slightly active against pinworms in mice.[6] These results were insufficient to warrant further investigation. Two compounds (**4** and **10**, Table II) showed *in vitro* activity against *Mycobacterium tuberculosis* and *Streptococcus pyogenes*, respectively. *In vivo* tests in mice against the experimental infections failed to show chemotherapeutic activity. *In vitro* activity against *Endamoeba histolytica* was observed with **14** (Table III).[7] In view of the fact that this compound was amebicidal at only the highest concentration, *in vivo* tests were not carried out. Compound **14** (Table III) was also slightly active at cytotoxic levels against *Trypanosoma cruzi* in chick embryo tissue cultures.[8,9] No activity was observed against the experimental infection in mice.[10] Compound **4** (Table II) exhibited no action against convulsions produced by electroshock,[11] but did exhibit moderate activity against convulsions induced by pentylenetetrazole.[12] None of the compounds reported in the work showed significant antitumor activity.[13]

(1) Supported in part by a grant (CY3908) from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare, Bethesda, Md., a Faculty Grant from North Texas State University, and a Parke, Davis and Co. Fellowship.

(2) J. R. Hoover and A. R. Day, *J. Am. Chem. Soc.*, **76**, 4148 (1954).

(3) P. Truitt, F. M. Wood, Jr., and R. L. Hall, *J. Org. Chem.*, **25**, 1160 (1960).

(4) K. Fries and K. Billig, *Ber.*, **58**, 1128 (1925).

(5) The physiological testings were arranged by Dr. E. Eslager of Parke, Davis and Company.

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(7) P. E. Thompson, J. W. Reinertson, H. A. McCarthy, A. Bayles, and A. R. Cook, *Antibiot. Chemotherapy*, **5**, 433 (1955).

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(11) J. E. P. Toman, E. A. Swinyard, and L. S. Goodman, *J. Neurophysiol.*, **9**, 23 (1946).

(12) C. M. Chen and C. R. Ensor, *Arch. Neurol. Psychiat.*, **63**, 56 (1950).

(13) The antitumor testings were arranged by Dr. Joseph Leiter of CCNSC and the complete results will be reported elsewhere.