Pyrimidine Derivatives with Antiamoebic Activity

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Received June 26, 1968

A series of new 2,6-disubstituted-4-chloropyrimidines was prepared in a program directed toward obtaining compounds having potential antiprotozoal activity. One member, 4-chloro-2-(*m*-chlorophenyl)-6-(2-dimethylaminoethoxy)pyrimidine (IIId), was found to have one-fourth the activity of emetine hydrochloride when tested *in vitro*. Attempts to enhance this activity by various structural modifications of the substituents on the pyrimidine nucleus either greatly reduced or completely eliminated the desired effect. These compounds and their screening results are summarized in Table I, the assays being expressed as the minimal inhibitory concentrations required to inhibit *Endamoeba histolytica*.[1]

Chemistry.—The pyrimidines under discussion were prepared by the reaction of a 2-substituted ethanol, ethanethiol, or ethylamine with appropriately substituted 4,6-dichloropyrimidines (see Scheme I). The latter pyrimidine intermediates were prepared by treating the corresponding 4,6-dihydroxypyrimidines with phosphorus oxychloride.

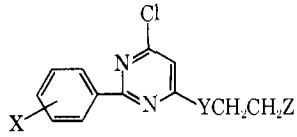
Experimental Section[2]

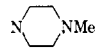
2-Substituted 4,6-Dihydroxypyrimidines.—4,6-Dihydroxy-2-phenylpyrimidine (Ia),[3] 2-(*p*-chlorophenyl)-4,6-dihydroxypyrimidine (Id),[4] and 4,6-dihydroxy-*p*-tolylpyrimidine (If)[3] have been reported previously. 4,6-Dihydroxy-2-(*m*-chlorophenyl)pyrimidine (Ic) [mp 256–257.5°; *Anal.* ($C_{10}H_7ClN_2O_2$) C, H, Cl, N] and 4,6-dihydroxy-2-(*p*-methoxyphenyl)pyrimidine (Ib) [mp 299–300° dec; *Anal.* ($C_{11}H_{10}N_2O_3$) C, H, N] were synthesized from the reactions of diethyl malonate with the corresponding amidine hydrochlorides according to the literature method.[3] 2-(3,4-Dichlorophenyl)-4,6-dihydroxypyrimidine (Ie), obtained from 3,4-dichlorobenzamidine hydrochloride[5] and diethyl malonate, was used directly in the next step.

2-Substituted 4,6-dichloropyrimidines (IIa–f) were prepared by the reaction of $POCl_3$ with the corresponding 2-substituted 4,6-dihydroxypyrimidines according to the literature procedure.[3] Yields and physical data of three compounds are listed in Table II. Compounds IIa, IIb, and IId have been reported in the literature.[3,4]

2,6-Disubstituted 4-chloropyrimidines (IIa–l) were made as exemplified by the preparation of 4-chloro-2-(3,4-dichlorophenyl)-6-[2-(dimethylamino)ethoxy]pyrimidine (IIIf). 4,6-Dichloro-2-(3,4-dichlorophenyl)pyrimidine (7 g) was added in small portions with gentle heating and stirring over a period of 5 min to 25 ml of 2-dimethylaminoethanol. An exothermic reaction took place during the addition. The heating and stirring were continued for an additional 6 min, and the reaction mixture was poured into H_2O (700 ml) with stirring. The oil which first separated

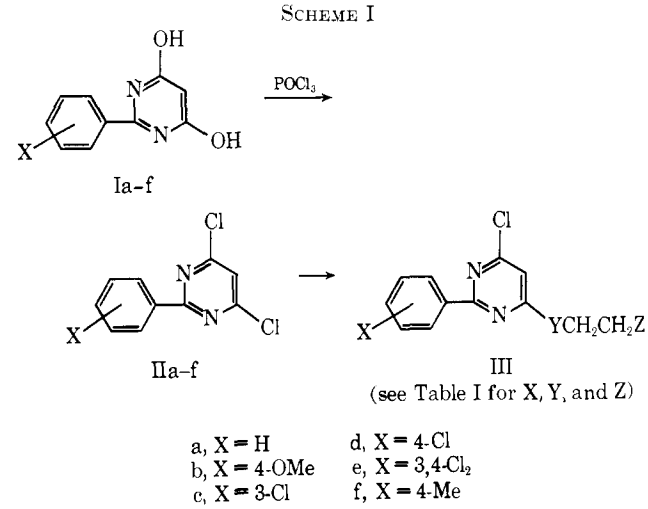
TABLE I



Compd III	X	Y	Z	Mp, °C	Formula[b]	Min inhib concn,[a] μg/ml Test substance	Emetine·HCl
a	H	O	H	62–64	$C_{12}H_{11}ClN_2O$	1000	2
b[c]	H	O	$N(Me)_2$	198–200	$C_{14}H_{16}ClN_3O \cdot HCl$	250	4
c	4-OMe	O	$N(Me)_2$	58–61	$C_{15}H_{18}ClN_3O_2$	1000	2
d	3-Cl	O	$N(Me)_2$	63–65	$C_{14}H_{15}Cl_2N_3O$	16	4
e	4-Cl	O	$N(Me)_2$	55–57	$C_{14}H_{15}Cl_2N_3O$	250	2
f	3,4-Cl_2	O	$N(Me)_2$	83.5–86.5	$C_{14}H_{14}Cl_3N_3O$	250	2
g[c]	3-Cl	S	$N(Et)_2$	220.5–223	$C_{16}H_{19}Cl_2N_3S \cdot HCl$	250	2
h	3,4-Cl_2	NH	$N(Me)_2$	99.5–102	$C_{14}H_{15}Cl_3N_4$	125	2
i	4-Cl	NH	$N(Me)_2$	78–80.5	$C_{14}H_{16}Cl_2N_4$	1000	4
j	4-Cl	NH	OMe	64–65	$C_{13}H_{13}Cl_2N_3O$	>1000	2
k	4-Me			93–95	$C_{16}H_{19}ClN_4$	1000	4
l	H	NH	OMe	48.5–50	$C_{13}H_{14}ClN_3O$	1000	2

[a] The test substance was incorporated and diluted in the aqueous phase of a modified Boeck-Drbohlav diphasic medium fortified with rice starch. The medium was inoculated with polybacteria and a known number of trophozoites of *E. histolytica* NIH 200. After 48 hr of incubation at 35°, the trophozoites were counted. Emetine hydrochloride was used as the standard. [b] All compounds were analyzed for C, H, Cl, N except compounds IIIj and l, which were analyzed for C, H, N. [c] Isolated and tested as the hydrochloride.

SCHEME I



a, X = H; b, X = 4-OMe; c, X = 3-Cl; d, X = 4-Cl; e, X = 3,4-Cl_2; f, X = 4-Me

(see Table I for X, Y, and Z)

(1) P. E. Thompson, D. A. McCarthy, A. Bayles, J. W. Reinertson, and A. R. Cook, *Antibiot. Chemotherapy*, **6**, 337 (1956).

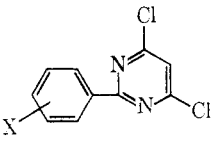
(2) Melting points were taken on a Thomas–Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Mr. B. Hofmann and associates of Wyeth Laboratories. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(3) J. A. Hendry and R. F. Homer, *J. Chem. Soc.*, 328 (1952).

(4) J. S. Moffatt, *ibid.*, 1603 (1950).

(5) P. Oxley, M. W. Partridge, T. D. Robson, and W. F. Short, *ibid.*, 763 (1946).

TABLE II



Compd	X	Mp, °C	Yield, %	Formula[a]
IIe	3-Cl	118.5–119	88	$C_{10}H_5Cl_3N_2$
IIe	3,4-Cl_2	122–124	90	$C_{10}H_4Cl_4N_2$
IIf	4-Me	84–85	75	$C_{11}H_8Cl_2N_2$

[a] All compounds were analyzed for C, H, Cl, N.

crystallized on standing. This material was collected on a filter and washed (H_2O) several times to give 8 g (95%) of product, mp 78–82°. Two recrystallizations from heptane afforded an analytical sample (Table I).

Acknowledgment.—The authors are indebted to Messrs. W. B. Edwards III and R. A. Fieber for their technical assistance and to Dr. Sanford B. Rosenman for the antiamoebic assays.

O-Demethyldecarbamoylnovobiocin

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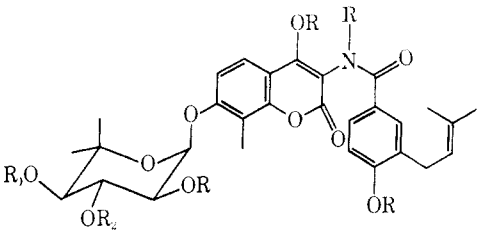
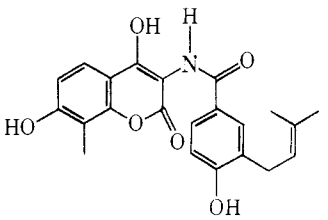
Received July 15, 1968

Novobiocin[1] (I), a crystalline antibiotic produced by *Streptomyces niveus*, is of commercial importance. One of the major by-products from the production of I is a biologically inactive material designated compound X. This compound has a uv spectrum identical with I but it has more polar chromatographic characteristics. Because of its apparent similarity and its relevance to the biosynthesis of novobiocin,[2] its structure was determined and is reported in this note.

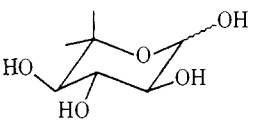
Novobiocin (I) can be isolated by extraction of the filtered fermentation broth with BuOAc. The antibiotic is then extracted into an aqueous buffer at a high pH. This solution is acidified and back-extracted into BuOAc. In this procedure compound X is accumulated in the spent aqueous fraction. It was extracted with EtOAc. The crude material obtained after removal of EtOAc showed five spots on paper chromatography, decarbamoylnovobiocin (II), isonovobiocin, compound X, and a more polar component, in order of decreasing R_f. Removal of the carbamoyl group by treatment with alkali converted this material into a mixture of two compounds, decarbamoylnovobiocin and compound X (spot position unchanged by the saponification). Compound X was obtained pure by countercurrent distribution and by silica gel chromatography. It was crystallized to give material of mp 124–126°; uv spectrum (EtOH), 207 mμ (log ε 4.73), 332 mμ (log ε 4.39); ir, 1685 cm^{-1} (amide C=O), no urethan C=O; nmr (DMF), τ 8.67 and 8.82 (6 H, $>CMe_2$), 8.23 (6 H, CMe_2), 7.71 (3 H, aryl Me), and no OMe signal. *Anal.* Calcd for $C_{29}H_{33}NO_{10}$: C, 62.69; H, 5.99; N, 2.52. Found: C, 62.67; H, 6.12; N, 2.47.



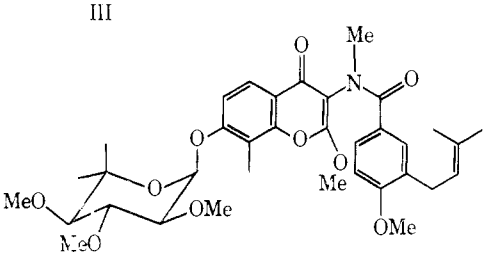
	R_1	R_2	R
I	Me	H_2NCO	H
II	Me	H	H
VI	Me	Me	Me
VII	H	H	H



III



IV



V

These data show that compound X does not contain a carbamoyl group or an O-methyl group. As a working hypothesis we assumed that the sugar moieties of compound X and II were identical except that X contained an OH group where II contained a CH_3O group. The chemical conversions described below of compound X showed this to be true.

Compound X was degraded following procedures[1] used in the structural elucidation of I. Acidic treatment gave two products: a crystalline aglycon, identical by ir spectroscopy with authentic novobiocic acid (III), a known degradation product of I, and an oily ethyl glycoside. This glycoside was shown to contain a *gem*-dimethyl group; by oxidation with H_2CrO_4 it yielded acetone collected as its 2,4-DNP. The glycoside was acetylated to give an oily triacetate verified by nmr. *Anal.* Calcd for $C_{15}H_{24}O_8$: C, 54.21; H, 7.28. Found: C, 54.26; H, 7.46. Further acid treatment of the glycoside yielded a free sugar which reacted with 3.3 equiv of IO_4^-, thereby indicating four vicinal OH groups. These data are consistent with noviose (IV), the sugar moiety which is present in II.

To prove that the configuration of the sugar moiety of X is identical with that of I, both X and II were converted separately to identical permethylated derivatives. Treatment of II with excess CH_2N_2 gave a mixture of two major products. Conversion of this mixture to two pentamethyl derivatives was accomplished by treatment with NaH–MeI in DMF at room temperature. The two major components were separated by alumina chromatography to give a 16% yield of crystalline material, mp 149–151°, and a 37% yield of noncrystalline material. Nmr, ir, and uv spectra support

(1) J. W. Hinman, E. L. Caron, and H. Hoeksema, *J. Am. Chem. Soc.*, **79**, 3790 (1957).

(2) L. A. Kominek in "Antibiotics," Vol. II, D. Gottlieb and P. D. Shaw, Ed., Springer-Verlag, New York, N. Y., 1967, p 253.