prepared for each compound. Colchicine (1:10,000–1:10,000,000 w/v) was run concomitantly with unknown compounds as standards.

At the termination of incubation, cells were assayed microscopically for the morphology of cells in the metaphase block. Metaphase arrest was characterized by round, chunky cells and was easily distinguished from the diamond shape of normal cells. The end-point was taken as the minimum concentration that produced over 50% of cells blocked in the metaphase.

Extraction and Preliminary Fraction of H. verticillata *Jacq.*—Coarsely milled leaves and stems of *H. verticillata* Jacq.[3] (100 g.) were extracted at room temperature with four 500-ml. portions of 95% ethanol. The extracts were combined, and solvent was removed under reduced pressure. The dark residue (4.2 g.) was partitioned between chloroform–water. The aqueous fraction had marginal antimitotic activity and was discarded. The chloroform-soluble material was repartitioned between heptane and 70% aqueous methanol. Negligible antimitotic activity was found in the residue from the heptane layer. Removal of solvent from the aqueous methanol layer gave 1.4 g. of a light-green residue containing greater than 95% of the antimitotic activity of the original extract.

Isolation of 4′-Demethyldesoxypodophyllotoxin and β-Peltatin—The residue from the aqueous methanol–heptane partitioning experiment was dissolved in 5 ml. of chloroform and placed on a magnesia-silica gel[4] column (50 g.). The column was eluted with increasing concentrations of methanol in chloroform. The desired materials, as determined by antimitotic activity, were present in the 2 and 5% methanol in chloroform fractions. Combination of these fractions (homogeneous by TLC) and removal of the solvent gave 166 mg. of active material.

The residue from absorption chromatography was dissolved in a minimum of benzene and placed on a partition column (40 g. of diatomaceous earth[5]; 50% v/w formamide as the stationary phase). The column was developed with benzene saturated with formamide. Fractions (3 ml.) were collected automatically on a Gilson linear fraction collector. Bioassay indicated that active materials were present in fractions 5–20 and 58–90.

Fractions 5–20 were pooled and solvent was removed under reduced pressure. The residue was crystallized from ethyl acetate–heptane (49 mg., 0.05%), m.p. 251–253° [lit. (13) m.p. 247°]. Comparison with an authentic sample established identity of the material as 4′-demethyldesoxypodophyllotoxin.

Fractions 58–90 were pooled and evaporated to dryness. The residue was crystallized from ethyl acetate–heptane (12 mg., 0.012%), m.p. 234.5–236°. Identity of the material as β-peltatin was established by comparison of the IR, mass, and NMR spectra with an authentic sample. Melting points were identical and mixture melting point was undepressed.

[3] A voucher sample of *H. verticillata* Jacq. is maintained at the Department of Botany, University of the West Indies, Kingston, Jamaica.

[4] Florisil, The Floridin Co., Pittsburgh, Pa.

[5] Celite, The Johns-Manville Co., New York, N. Y.

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Received October 19, 1970.

Accepted for publication December 18, 1970.

I wish to thank Dr. I. Cornman and Dr. Dennis Adams, University of the West Indies, Kingston, Jamaica, for collection and identification of the sample of *H. verticillata* used in this study. I also thank Dr. A. von Wartburg, The Sandoz Company, Basel, Switzerland, for a sample of 4′-demethyldesoxypodophyllotoxin, and Mr. George Motoasca, S. B. Penick Company, New York, N. Y., for a sample of β-peltatin.

Synthesis and Antibacterial Activity of 5-Nitro-2-furaldehyde Phenylhydrazones

Keyphrases ☐ 5-Nitro-2-furaldehyde phenylhydrazones—synthesis ☐ Antibacterial activity—5-nitro-2-furaldehyde phenylhydrazones

Sir:

The antibacterial activity of 5-nitro-2-furaldehyde derivatives has been known for some time (1–3). Although many of them have been synthesized, their phenylhydrazones have not been studied.

To test their *in vitro* activity, a series of new compounds with this structure has been prepared. The phenylhydrazones were obtained in good yield by reaction of 5-nitro-2-furaldehyde with substituted phenylhydrazines (Table I). The phenylhydrazines were prepared as described by Hunsberger *et al.* (4).

Although many of the substituted phenylhydrazones showed antibacterial activity at high concentrations, only Compound 1 had a broad spectrum activity (Table

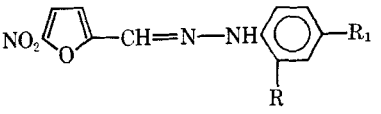

Table I—5-Nitro-2-furaldehyde Phenylhydrazones[a]

Number[b]	R	R_1	Yield, %	M.p.[c]	Formula	Anal., %[d] Calcd.	Found
1	H	F	91	171–173°	$C_{11}H_8FN_3O_3$	C, 52.96 H, 3.23 N, 16.86	C, 53.18 H, 3.31 N, 16.59
2	H	Cl	70	194–196°	$C_{11}H_8ClN_3O_3$	C, 49.73 H, 3.03 N, 15.81	C, 50.00 H, 3.25 N, 16.00
3[e]	H	Br	75	185–186°	$C_{11}H_8BrN_3O_3$	C, 42.59 H, 2.58 N, 13.55	C, 42.46 H, 2.28 N, 13.46
4	H	I	92	215–217°	$C_{11}H_8IN_3O_3$	C, 36.99 H, 2.25 N, 11.76	C, 36.98 H, 1.96 N, 11.80
5	H	CH_3	70	165–167°	$C_{12}H_{11}N_3O_3$	C, 58.77 H, 4.52 N, 17.13	C, 58.50 H, 4.30 N, 16.94
6	H	OCH_3	92	188–190°	$C_{12}H_{11}N_3O_4$	C, 55.17 H, 4.24 N, 16.08	C, 54.95 H, 4.42 N, 15.89
7	H	COOH	90	240° dec.	$C_{12}H_9N_3O_5$	C, 52.37 H, 3.30 N, 15.27	C, 52.60 H, 3.51 N, 15.10
8	F	H	75	165–167°	$C_{11}H_8FN_3O_3$	C, 52.96 H, 3.23 N, 16.86	C, 53.21 H, 3.50 N, 17.02
9	Cl	H	75	168–169°	$C_{11}H_8ClN_3O_3$	C, 49.73 H, 3.03 N, 15.81	C, 49.60 H, 3.42 N, 15.90
10	Br	H	95	148–150°	$C_{11}H_8BrN_3O_3$	C, 42.59 H, 2.58 N, 13.55	C, 42.85 H, 3.02 N, 13.55
11	I	H	90	146–148°	$C_{11}H_8IN_3O_3$	C, 36.99 H, 2.25 N, 11.76	C, 37.24 H, 2.24 N, 11.75
12	CH_3	H	93	168–169°	$C_{12}H_{11}N_3O_3$	C, 58.77 H, 4.52 N, 17.13	C, 58.80 H, 4.29 N, 16.83
13	OCH_3	H	70	148–150°	$C_{12}H_{11}N_3O_4$	C, 55.17 H, 4.24 N, 16.08	C, 54.95 H, 4.53 N, 15.80
14	COOH	H	95	250° dec.	$C_{12}H_9N_3O_5$	C, 52.37 H, 3.30 N, 15.27	C, 52.60 H, 3.54 N, 15.38

[a] The UV, IR, and NMR spectra are in agreement with the proposed structures. [b] All compounds in this table were recrystallized from ethanol or ethanol–water. [c] Melting points are uncorrected. [d] Elemental analyses were performed by Mrs. M. Casanello. [e] Lit. (8) m.p. 201°.

II). Compounds 5–7 and 9–14 were active against *Bacillus subtilis* at concentrations of about 200–400 mcg./ml. The minimum inhibitory concentrations (M.I.C.) were determined by the cup method; the M.I.C. values for medium containing 10% human serum (5) were unchanged, except for the genera Salmonella which showed increased values (1200 mcg./ml.). Compound 1 exhibits a very low acute toxicity level compared with some clinically useful nitrofurans (1), although comparisons are rather difficult since the toxicity of nitrofurans is dependent on the crystal size (6). No deaths occurred when doses of 1000 mg./kg. were given to white mice, either orally or intraperitoneally.

Table II—Activity of Compound 1

Test Organism	M.I.C., mcg./ml.
Escherichia coli ATCC 9637	180
Pseudomonas aeruginosa ACM 69	240
Proteus vulgaris ATCC 13315	200
Salmonella typhimurium AMC ETS 9	400
S. paratyphi B (I. Malbran)	400
S. paratyphi C (I. Malbran)	400
Bacillus subtilis ATCC 6633	180
Shigella flexneri (I. Malbran) serotype 10	240

Stability tests performed with Compound 1, as described by Garrett and Carper (7), showed no appreciable decomposition, even during 30 days at 60°, when protected from light.

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Received July 27, 1970.
Accepted for publication October 30, 1970.

Cycloheptaamylose Inclusion Complexes of Barbiturates: Correlation between Proton Magnetic Resonance and Solubility Studies

Keyphrases □ Cycloheptaamylose inclusion complexes—barbiturates □ Proton magnetic resonance—analysis

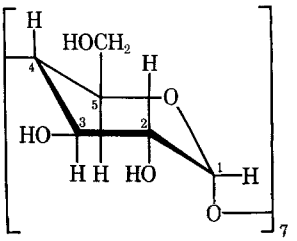
Sir:

In the past few years, Lach and his coworkers (1–5) reported extensively on the complexation of cycloamyloses (cyclodextrins) with various medicinally useful molecules. These studies, based upon the solubility method of Higuchi and Lach (6), are indicative of stereospecific interactions between drug and cycloamylose molecules; however, they do not provide *direct* evidence for inclusion of the solute within the cycloamylose cavity. Elucidation of the mechanism of such interactions, therefore, remains largely speculative.

We recently described a proton magnetic resonance (PMR) method for examining the mode of interaction of cycloheptaamylose with a variety of aromatic substrates (7). Our method is based upon the rationale that when an aromatic moiety is included within the cavity of the doughnutlike cycloheptaamylose molecule (I)[1], protons located within the cycloheptaamylose cavity (H-3, H-5, and, possibly, H-6) undergo appreciable shielding due to the anisotropy of the aromatic moiety, whereas protons located at the exterior of the torus (H-1, H-2, and H-4) are relatively unaffected. We have now extended our studies to complexation of some pharmacologically active barbituric acid derivatives (II). In the present communication, we demonstrate a correlation between results obtained by the conventional solubility method and by our PMR method.

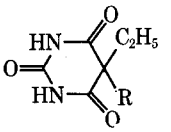
Formation constants (K_f), determined by the solubility method, and the substrate-induced chemical shift changes ($\Delta\delta$) for a variety of cycloheptaamylose–barbiturate complexes are listed in Table I. It is evident from the results shown in this table that a parallel trend exists between K_f and $\Delta\delta$; *i.e.*, K_f and $\Delta\delta$ for H-5 are of the order: barbital < amobarbital < pentobarbital < phenobarbital. From a comparison of the relative magnitudes of $\Delta\delta$ for H-5 and H-3, it appears that association takes place by approach of the barbiturate from the primary hydroxyl side of cycloheptaamylose. For phenobarbital, $\Delta\delta$ for H-5 is the largest, which indicates that the anisotropic moiety (the phenyl side chain) of this barbiturate penetrates the cycloheptaamylose cavity. This penetration is apparently rather shallow when compared with that previously observed for simple aromatic substrates (7), since $\Delta\delta$ for H-3 is minimal. For the other barbiturates also, $\Delta\delta$'s for H-5 are significant and positive. This observation would be consistent with the well-recognized upfield shifts due to hydrophobic interactions. Such interactions could result from inclusion of the nonpolar, aliphatic side chains of the barbiturates within the cavity.

The fact that barbital, amobarbital, and pentobarbital, with their respective ethyl, isopentyl, and *n*-pentyl side chains, show a corresponding order in the $\Delta\delta$ of H-5 and in the formation constants supports the suggested mode of interaction. The formation constant for phenobarbital is the highest, an apparent consequence of the snug fit of the phenyl ring relative to the aliphatic side chains of the other barbiturates. Hydrogen bonding between the heterocyclic barbiturate nucleus and the primary hydroxyl groups of cycloheptaamylose also could be partly responsible for the interaction. Such a possibility would not be precluded by the suggested hydrophobic interaction.



cycloheptaamylose

I



	R
barbital	$-CH_2-CH_3$
amobarbital	$-CH_2-CH_2-CH(CH_3)_2$
pentobarbital	$-CH(CH_3)-CH_2-CH_2-CH_3$
phenobarbital	$-C_6H_5$

barbiturates

II

[1] The constituent glucose units of cycloamyloses are known to have the Cl chair conformation (8–10). Space-filling molecular models (Corey–Pauling–Koltun) show that the interior of the cycloheptaamylose cavity has, from the primary hydroxyl side, successive layers of H-6, H-5, the ring oxygens, and H-3.