The agreement between the experimental HLB values of a series of sorbitan derivatives and the values calculated by Eq. 1 was good (Table I).

It is shown in this communication that for a different category of nonionic surfactants, namely, polyoxyethylated octylphenols, $C_8H_{17}C_6H_4O(C_2H_4O)_pH$, Eq. 1 does not apply. These are homogeneous or single-species surfactants; each compound has a single value of p (3). Because experimental HLB determinations of these surfactants have not been made, their HLB values were calculated by

$$\text{HLB} = \frac{E}{5} \qquad \text{(Eq. 2)}$$

where E is the percentage of polyoxyethylene in the surfactant molecule. For polyoxyethylated octylphenols, Eq. 2 can be rewritten as

$$\text{HLB} = \frac{4405p}{5(206.32 + 44.05p)} \qquad \text{(Eq. 2a)}$$

where 206.32 is the molecular weight of octylphenol. Equation 2 was derived by Griffin from experimental measurements and is reported to be applicable to all polyoxyethylated nonionic surfactants (2); this is the basis of the HLB system for rating these surfactants. The HLB values calculated according to Eq. 2 are those listed in a recent compilation (2) and are considered to be correct.

These HLB values are compared in Table I with the HLB values calculated from the distribution coefficients (3) according to Eq. 1 for several surfactants of the homologous series. The correct HLB values (experimental or calculated by Eq. 2) for the entire series, as well as for the sorbitan-based surfactants, are plotted in Fig. 1 against the logarithms of the distribution coefficients. The curve for the polyoxyethylated octylphenols diverges considerably from the straight line of the sorbitan-based surfactants. A universal correlation between the distribution coefficients and the HLB, applicable to all nonionic surfactants, requires that the two curves coincide. An alternative, although less likely, explanation for the divergence is that Eq. 2 is not applicable to all nonionic surfactants; specifically, that it breaks down for homogeneous surfactants.

The distribution coefficients of normally distributed polyoxyethylated octylphenols (3, 4), where each surfactant has a range of p values, are also plotted against the HLB in Fig. 1. A broadened molecular weight distribution of the nonionic surfactants affects the relationship between HLB and distribution coefficient far less than a change in their chemical characteristics.

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HANS SCHOTT

Temple University School of Pharmacy
Philadelphia, PA 19140

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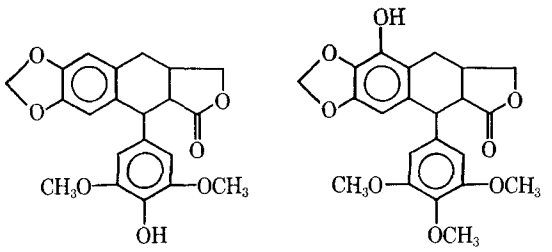
Isolation and Characterization of Cytotoxic Principles from *Hyptis verticillata* Jacq.

Keyphrases ☐ *Hyptis verticillata*—cytotoxic principles ☐ β-Peltatin, 4′-demethyldesoxypodophyllotoxin—isolation, identification, *H. verticillata* ☐ Antimitotic activity—*H. verticillata* extracts ☐ IR spectrophotometry—identification ☐ NMR spectroscopy—identification ☐ Mass spectroscopy—identification

Sir:

A recent observation (1) that aqueous extracts of *Hyptis verticillata* Jacq. (Labiatae) inhibited development of sea urchin eggs led to a systematic study of extractable substances from this source. As a result of this study, two antimitotic agents, 4′-demethyldesoxypodophyllotoxin (I) and β-peltatin (II) were isolated and identified by comparison with authentic materials.

The ubiquity of lignans related to the 2,3-dimethylphenylnaphthalenes (2–10) is well documented, and the breadth of occurrence of cytotoxic substances related to podophyllotoxin is continually expanding (11–12). Isolation of these materials from *H. verticillata* represents, to our knowledge, the first report of their occurrence in the Labiatae family and attests to their widespread occurrence.



I II

Experimental[1]—Melting points were determined on a Hoover-Thomas capillary melting-point apparatus. IR spectra were determined in chloroform solution on a Beckman IR-8. NMR spectra were determined on a Varian A-60 spectrometer. Mass spectra were determined on a Hitachi RMU-6H mass spectrometer. Antimitotic activity of the fractions and materials obtained as a consequence of purification was determined[2] using Earle's L929 mouse fibroblast cell line as the test system.

Antimitotic Assay—Earle's L929 mouse fibroblast cells (1.5×10^5 cells/tube) were incubated 10 hr. at 36° in culture tubes, each tube containing 1 ml. of Eagle's minimum essential medium supplemented with 10% calf serum. To pairs of tubes containing the incubated cells was added 0.1 ml. of the test solution containing the suspected antimitotic agent. The cells were incubated an additional 18–24 hr. Generally, three to four dilutions varying by factors of 10 were

[1] Microanalyses were performed by Mr. Malcolm Stone of the A. H. Robins Co.

[2] By Dr. R. W. Tankersley, A. H. Robins Co.

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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VICTOR F. GERMAN

A. H. Robins Company
Richmond, VA 23220

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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