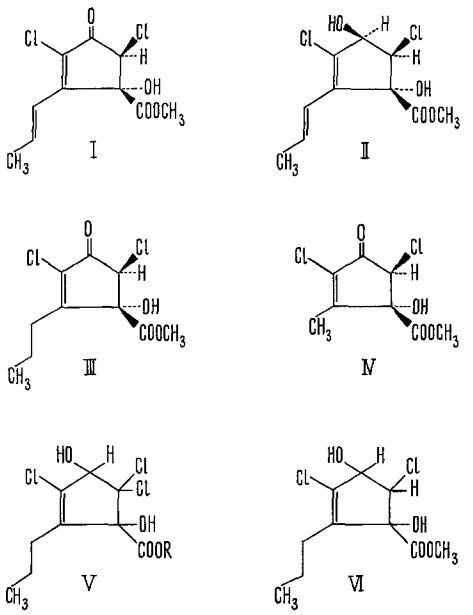
Synthesis of (±) Dihydrocryptosporiopsin

The dichlorocyclopentenone I is a metabolite, possessing antifungal activity, produced by the coprophilous fungus *Sporormia affinis* Sacc., Bomm and Rouss[1] and by a *Cryptosporiopsis* sp.[2], an imperfect fungus isolated from yellow birch, *Betula alleghaniensis* Britt. The name cryptosporiopsin has been advanced for I[2a]. Isolation of the related metabolite II from *Periconia macrospinosa* has recently been reported[3].

Hydrogenation of cryptosporiopsin in ethyl acetate solution with a palladium-charcoal catalyst gives rise to a dihydro-product III, mp 90–96°, in which the *trans*-allyl side chain is reduced to a *n*-propyl group[2b,c]. In a previous paper[4], we have described the conversion of *m*-cresol to the dichlorocyclopentenone IV, suggesting that it should be feasible to synthesize cryptosporiopsin derivatives by hypochlorite-induced rearrangement of appropriately substituted phenols[5–7]. We now report the successful application of the hypochlorite-phenol rearrangement in the synthesis of racemic dihydrocryptosporiopsin III.



Meta-propyl phenol (prepared by reduction of isosafrole with sodium in alcohol[8]) was treated with chlorine in alkaline solution under conditions based on earlier studies[4–7]. The hydroxy acid V (R=H) could be isolated from the mixture of acidic products by partition chromatography on silica gel[9]. Alternatively, the methyl ester V ($R=CH_3$) could more readily be obtained in a pure state, mp 144–145°[10], on chromatography of the mixture resulting from treatment of the hypochlorite products with diazomethane. For the purposes of the synthesis, it was found expedient to effect purification at a later stage. Accordingly the crude acidic mixture was treated with sodium amalgam to effect reduction of the *gem*-dichloro grouping in V (R=H)[4–7]. Following esterification with diazomethane, chromatography afforded the desired dichlorodihydroxy ester VI[11], mp 178–180° (1.5% overall yield from *m*-propyl phenol).

Finally, conversion of VI to racemic dihydrocryptosporiopsin III, mp 101–103°, was accomplished quantitatively by oxidation with Jones' reagent in acetone. The identity of synthetic and naturally derived material was established by IR-, UV-, nuclear magnetic resonance and mass-spectrometry.

Zusammenfassung. Die Synthese von (±) Dihydrocryptosporiopsin aus 3-Propylphenol wird beschrieben.

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Canada Department of Fisheries and Forestry, Forest Research Laboratory, Fredericton (New Brunswick, Canada), 12 January 1970.

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[10] All compounds for which melting points are reported gave satisfactory analytical results by microanalysis and/or mass-spectrometry.

[11] The observed coupling constant (6.4 Hz) of the *vicinal* protons at carbons 4 and 5 of VI[3,6,7], in conjunction with the established stereochemistry of III, its oxidation product (vide infra), suggests that the relative stereochemistry of VI and the *Periconia* metabolite (II) may be identical. In accord with this tentative assignment, BURGSTAHLER[7] has suggested, on the basis of different evidence, that the *phenol*-hypochlorite product is probably a *trans*-diol.

Identification of the Drug Darvon and its Metabolites in the Urine of a Comatose Patient Using a Gas Chromatograph-Mass . Spectrometer-Computer System[1]

High- and low-resolution mass-spectrometry, in conjunction with data obtained from a gas-chromatograph low-resolution mass-spectrometer computer system, were used in the analysis of urine from a patient suspected of ingestion of an overdose of Librium[2]. No indication of this drug could be found in the urine, but instead the drug Darvon (IV)[3] could be detected, together with several of its metabolites.

In patients suffering from drug overdose, therapy is best administered with a thorough knowledge of the

causative agent. This information must be unambiguous and available as rapidly as possible. Recognizing this need a cooperative program between this laboratory and Prof. HEDLEY-WHYTE of Beth Israel Hospital and the Harvard Anesthesia Center, Boston (Mass.), was initiated to develop techniques for the rapid identification of drugs in body fluids.

Structural identification of drugs and their metabolites, superimposed upon the inherent complexity of body fluids, may demand the ultimate in sensitivity of detection. Such capability can, in approximately 20% of patients comatose after ingestion of unknown drugs, be provided only by a mass-spectrometric analysis of gas-chromatographic effluents. Comparison of gas-chromatographic retention times is satisfactory in the other 80% of patients comatose after ingestion of drugs.

In this laboratory a gas-chromatograph low-resolution mass-spectrometer computer system[4], in conjunction with conventional high- and low-resolution data, has been employed for the analysis of body fluid extracts. This approach has proven very successful in a number of instances and has often provided information on the metabolites of the drug[5].

The computer data acquisition system presently employed generates spectra from the mass-spectrometer, assists in spectra interpretation, and allows a computerized search of mass-spectra contained in a library which currently consists of approximately 8000 mass-spectra stored on magnetic disk[4–6].

In this particular instance, a 24-year-old woman was admitted to Beth Israel Hospital, Boston, in critical condition, suspected of ingesting an overdose of the drug Librium. A urine sample (200 ml) was extracted with dichloromethane to yield separately the acidic, neutral and basic fractions. Another portion (100 ml) was evaporated to dryness.

High- and low-resolution mass-spectra were obtained on each of the 4 fractions ('neutrals', 'acids', 'bases', 'evaporated') by introduction of a sample directly into the ion source of the mass-spectrometer. Because Librium was suspected to be the drug taken, and because its metabolic products are known to retain the chlorine attached to the phenyl group[7], the high-resolution mass-spectra were analyzed for the presence of chlorine using previously described computer techniques[8]. None of the ions were found to contain chlorine, thus eliminating Librium as a suspect drug. The possibility of the presence of drugs containing sulfur, bromine, or silicon was also eliminated using the same approach.

Further examination of the spectra indicated that many abundant ions were derived from normal constituents of urine[9], such as urea, creatinine and hippuric acid. The presence of abundant ions in the mass-spectra which could not be derived from normal constituents of urine suggested that an additional drug or drugs might be present.

Consequently, a gas-chromatograph low-resolution mass-spectrometer computer system[4] was employed to obtain mass-spectra of the separated components. Mass-spectra of the effluent gas stream were determined every 3 sec during the entire chromatogram, and hence several spectra were generally obtained for each gas-chromatographic peak. The summed intensities of each consecutive spectrum are plotted in Figure 1 which thus represents the gas-chromatogram as generated by the computer.

A comparison of the mass-spectra obtained for the major peaks with the above-mentioned collection of mass-spectra by the computer, immediately identified peaks A and B as benzyl alcohol and cresol, respectively, and indicated that C and D were isomers of diphenylbutene. The 2

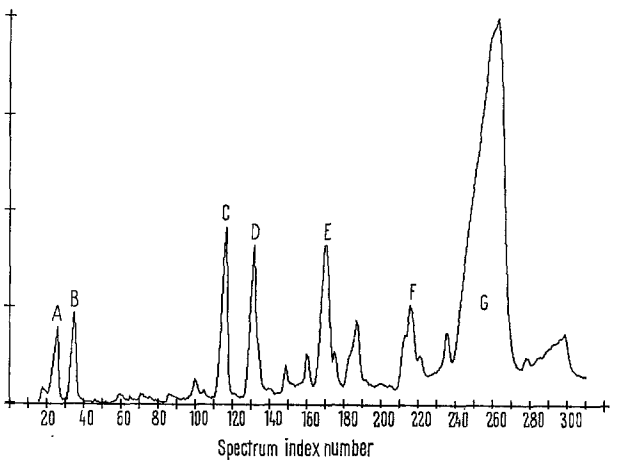

Fig. 1. Total ionization plot.

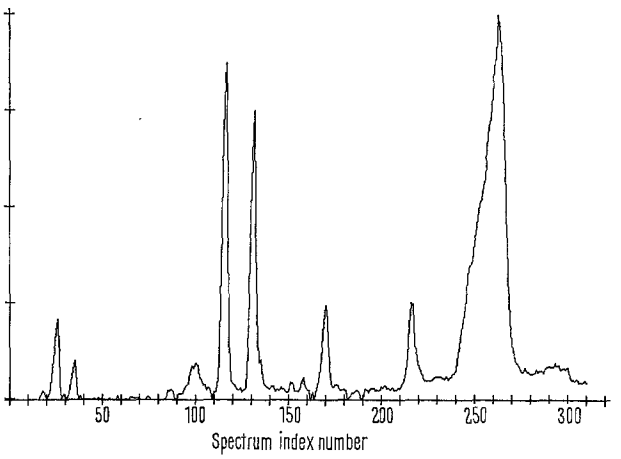

Fig. 2. Mass chromatogram *m/e* 91.

[1] This publication describes the identification of a drug causing coma in a patient. The drug was identified by analysis of the patient's urine performed at the NIH sponsored Special Research Resource Facility for Mass Spectrometry at M.I.T., under emergency conditions. Most of the experiments, measurements and interpretation reported had to be made within 1 day, thus requiring the simultaneous efforts of many members of M.I.T.'s mass-spectrometry group.

[2] Librium is the trade name for 7-chloro-2-methylamino 5-phenyl-3H-1,4-benzodiazapine-4-oxide (Hoffmann-La Roche, Inc., Nutley, N.J.).

[3] Darvon is the trade name for 4-dimethylamino-3-methyl 1,2-diphenyl-2-butanol propionate hydrochloride. The free base is commonly referred to as propoxyphene (Eli Lilly, Inc., Indianapolis, Ind.).

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last compounds are hardly normal constituents of urine but could possibly be metabolites of the drug one was searching for. If this is correct, the drug itself should be a derivative of diphenylbutene. To detect all such compounds a plot of *m/e* 91 (indicative of all compounds having a $C_6H_5CH_2$ moiety) in the consecutive mass-spectra were generated (Figure 2). It is clearly indicated that only peaks A–G represent mass-spectra with abundant peaks at *m/e* 91 and the attention was then focused on the spectra of fractions E, F, and G (see Figure 3). It was found that peaks C, D, and F were related by common ions at *m/e* 208, 193, 117, and 115; components E and F by common strong peaks at *m/e* 58 (C_3H_8N) and *m/e* 265; and peaks E and G by *m/e* 205 and 115, with component G lacking *m/e* 58.

The presence of an ion at *m/e* 208 in F seemed to indicate that F contains the same carbon skeleton as C and D. High-resolution data suggested the composition $C_{19}H_{23}N$ for the ion at *m/e* 265. This elemental composition, combined with the very intense peak at *m/e* 58 (C_3H_8N),

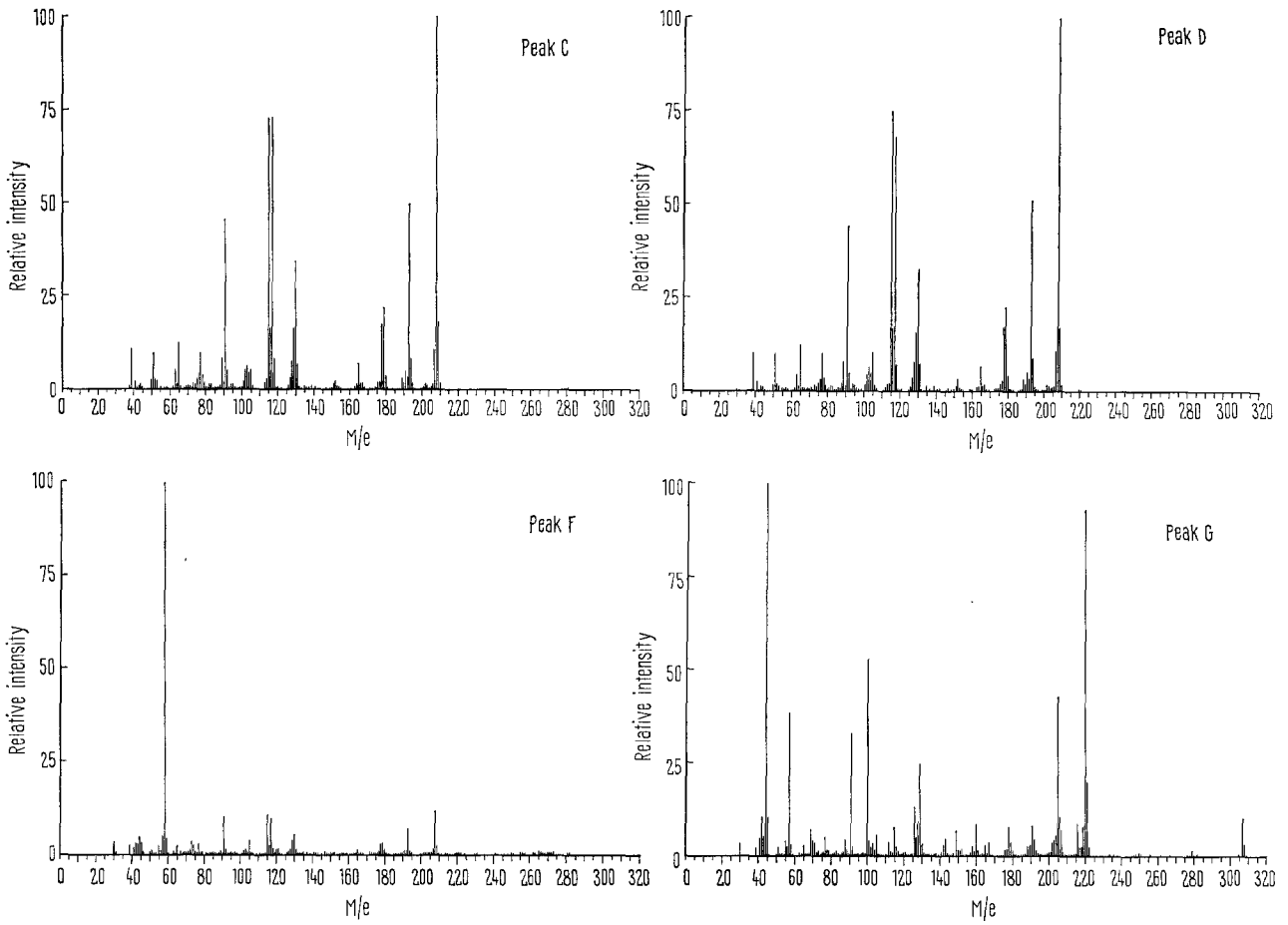
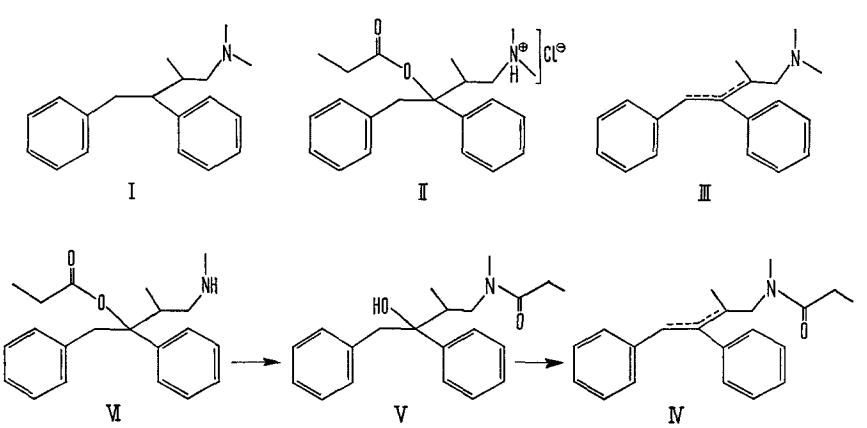


Fig. 3.



which is suggestive of a N,N-dimethylamino moiety, led to the postulation of skeleton I as a working hypothesis. This structure combines the observed data with the attractiveness of a structure closely related to the common analgesic, propoxphene hydrochloride (Darvon, II). A sample of propoxphene was then obtained and its mass-spectrum and retention time (coinjection) proved indeed to be identical to those of component F.

The suggested molecular composition of $C_{19}H_{23}N$ as well as the mass-spectrum of component E suggest structure III, the product of either thermal, electron impact or metabolic dehydration of the carbinol resulting from hydrolysis of the ester function in propoxyphene. The by far most abundant component of the extract is fraction G. Its mass-spectrum exhibited an ion at *m/e* 307 ($C_{21}H_{25}NO$) which apparently loses C_4H_9NO to form an ion at *m/e* 220. This indicates loss of both heteroatoms (N and O) along with 4 carbon atoms and implies structural proximity of the nitrogen and oxygen atoms in G compared to II. Furthermore, the most intense peak appears in this spectrum at *m/e* 44 rather than *m/e* 58 which requires the absence of one of the N-methyl groups. The most likely structure would be IV, the result of an intramolecular acyl migration in N-desmethyl propoxyphene (VI) followed by dehydration of the intermediate amide (V). Compound VI has indeed been found to be the major metabolite of propoxyphene hydrochloride in humans[10], and its facile conversion to (V) is also known[11,12].

The amount of time required for the identification of the drug causing the patients condition would have been considerably reduced if a more extensive library of mass-spectra of commonly encountered drugs and their metabolites had been available. The compilation of such a library is currently in preparation[13,14].

Zusammenfassung. Mit Hilfe von hoch- und niederauflösender Massenspektroskopie sowie Daten eines Gaschromatograph-Massenspektrometer-Computer-Systems wurden die Droge Darvon[3] sowie eine Reihe ihrer Metaboliten im Harn eines Überdosispatienten nachgewiesen.

J. R. Althaus, K. Biemann[15], J. Biller, P. F. Donaghue, D. A. Evans, H.-J. Förster, H. S. Hertz, C. E. Hignite, R. C. Murphy, G. Preti and V. Reinhold

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[15] To whom all inquiries should be addressed.

Distribution of Integumental Tyrosinase Activity in Red-Eared Turtle, *Pseudemys scripta elegans*

The integumental tyrosinase activity in 4 species of reptiles, *Trionyx ferox* (Florida softshell turtle), *Caiman sclerops* (spectacled caiman), *Opheodrys aestivus* (vine snake) and *Anolis carolinensis* (American chameleon), has been described[1,2]. In these species the tyrosinase activity of the dorsal skin is higher than that of the ventral skin. In 3 species[3] the subcellular distribution of tyrosinase activity follows the amniote pattern, i.e., tyrosinase activity is confined to the particulate fraction. However, in *Opheodrys aestivus* the tyrosinase activity occurs in both particulate and soluble fractions as in most anamniotes[3]. As previous studies have dealt with general integumental areas, a detailed investigation of the distribution of tyrosinase activity in a number of skin areas from another reptile, *Pseudemys scripta elegans* (red-eared turtle), was undertaken in order to more precisely evaluate the enzymic activity in anatomically diverse regions.

Materials and methods. 5 non-melanistic adult red-eared turtles, 3 males weighing 720, 851 and 964 g and 2 females weighing 785 and 907 g, were utilized. The animals were sexually quiescent. The animals were decapitated and the different skin areas (Table) were removed and frozen (−27 °C). The enzyme preparation, radiometric assay procedures and substrates utilized have been reported previously[4,5]. The activities of the enzyme preparations were DOPA dependent, completely inhibited by sodium diethyldithiocarbamate (6 m*M*) and stable for at least 2 weeks at 0–4 °C. The methods of protein analysis and statistical evaluation also have been presented previously[4]. Data are expressed in the form $\bar{X} \pm \sigma_{\bar{x}}$ except those of the red patches (head) as the patches of all animals were combined prior to assay. Approximately 2000 assays were performed.

Results and discussion. As sex differences in tyrosinase activity were not discernible, the data for each skin area were consolidated. The tyrosinase activity varied considerably in the different integumental areas studied (Table). High enzymic activities occurred in the head, tail,

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