duce dermal reactions: severe erythema, hyperkeratinization, and desquamation with marked induration and fissuring on the macroscopic level; inflammation with a number of polymorphonuclear and round cells in the dermis and acanthosis with hyperplasia and various degrees of necrosis in the epidermis on the microscopic level; and the highest increase in lipid phosphorus content.

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Reaction Gas Chromatography III

Recognition of Tropane Structure in Alkaloids

By CZESLAWA RADECKA and ISHWAR C. NIGAM

The technique of "reaction gas chromatography" involving dehydrogenation of heterocyclic compounds by a platinum-firebrick catalyst was applied to alkaloids possessing the tropane moiety in their structures. The reaction chromatogram of 3-tropanol, from which most of these alkaloids may be considered to be derived, exhibited several peaks, among which were those due to pyrrole, methylpyrrole, pyrrolidine, pyridine, piperidine, and toluene. These products were also produced by atropine, homatropine, scopolamine, ecgonine, and cocaine when these alkaloids were examined under similar experimental conditions. Atropine, homatropine, and scopolamine exhibited some additional peaks which were correlated with their structures.

APPLICATIONS OF reaction gas chromatography involving catalytic dehydrogenations, to alicyclic and heterocyclic compounds in general and to monoterpenoids in particular, were described in earlier publications from this laboratory (1, 2). The success achieved in experiments with heterocyclic compounds (1) prompted the authors to extend the technique to bases belonging to the tropane group of alkaloids. The present communication reports the results of the investigations. It was observed that these alkaloids as well as tropine, the alcohol from which they are derived, suffered pyrolytic dehydrogenation when they were passed through a reactor packed with 5% platinum on base-washed firebrick. Structures of the substances examined could be meaningfully correlated with the products of reaction and the technique may be employed for the detection of tropane moiety in natural and synthetic alkaloids.

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Previous paper: Mizrahi, I., and Nigam, I. C., *J. Chromatog.*, **25**, 230(1966).

EXPERIMENTAL

Apparatus—*Gas Chromatograph*—Burrell Kromotog K-2 equipped with thermal conductivity detector and gas valves for directing the carrier gas to the chromatographic column directly or *via* the reactor, as required.

Column—Glass tube (225 cm. long, 6 mm. i.d.)

packed with 20% Carbowax 20M on base-washed firebrick and maintained at 140 ± 1°.

Carrier Gas—Helium, 75 ml./min.

Reactor—Construction of the reactor has been described (1). It was filled with 0.7 Gm. of the catalyst and maintained at 280 ± 5° with the help of an external heating jacket. A thin platinum wire was inserted into the tapered end of the reactor leading into the column in order to prevent deposition of less volatile products.

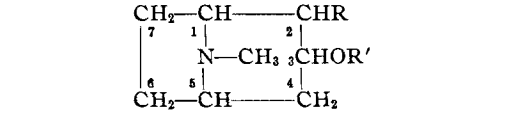
Catalyst—Five percent platinum on base-washed firebrick (30–60 mesh) prepared as described before (1).

Procedure—Ethanolic solutions of the substances were employed. Aliquots containing 1–3 mg. of the samples were used for injections. Substances existing in the liquid state at room temperature were used without solvent. The chromatographic peaks were tentatively identified by comparison of retention times with those of pure reference standard. Identifications of compounds possessing chromophoric groups were confirmed by collecting the corresponding effluent fractions in ethanol and recording the ultraviolet spectra.

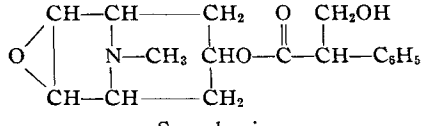
RESULTS AND DISCUSSIONS

Tropane alkaloids are derivatives of 3-tropanol formed by esterification of the hydroxyl group and/or by substitutions on other carbon atoms. The formulas of compounds used in the present investigations are shown in Table I. It may be

TABLE I—TROPANE DERIVATIVES



Compd.	R	R′
3-Tropanol	H	H
Atropine	H	—C(=O)—CH(CH₂OH)—C₆H₅
Homatropine	H	—C(=O)—CH(OH)—C₆H₅
Ecgonine	—COOH	H
Cocaine	—COOCH₃	—C(=O)—C₆H₅



Scopolamine

expected that the products of pyrolytic dehydrogenation of tropanol would be present among the products derived from the alkaloids. It was, therefore, considered worthwhile first to study the degradation of tropanol and then to employ the data for interpretation of results obtained by dehydrogenation gas chromatography of the alkaloids.

Tropanol—The reaction gas chromatogram of tropanol exhibited several peaks (Fig. 1,A). Apparently the fused ring system of tropanol had suffered cracking under the experimental conditions, leading to the formation of smaller molecules. This was confirmed by identification of some of the reaction products. The first step of the reaction may be the dehydration of tropanol to tropidine (3). Degradation of tropidine may then proceed *via* rupture of C—N or C—C bonds as indicated in Scheme I. The rupture of a C—N bond on passage of vapors of tropane over a palladium-asbestos catalyst at 300° was reported by Ehrenstein and Marggraff (4). Under more drastic conditions—*viz.*, fusion with alkali—the nitrogen atom was completely detached in the form of methylamine (3, 5). The rupture of C—C bonds of the tropane skeleton has also been observed. Thus when tropidine was dehydrogenated with bromine, 3,5-dibromopyridine, ethylene dibromide, and methyldibromopyridine were formed (6). Although the presence of pyrrole derivatives among the products of the above degradations was not reported, their formation under conditions of drastic degradation is quite likely to take place since it would involve only rupture of C—C bonds without affecting the N—CH_3 group (4). The rupture of the fused ring system of tropanol may, therefore, give rise to three types of monocyclic systems: a five-membered heterocyclic ring, a six-membered heterocyclic ring, and a seven-membered carbocyclic ring.

As expected, *N*-methylpyrrole, pyridine, and pyrrole were readily identified among the products as peaks No. 4, 5, and 9, respectively. (Table II.) Peak No. 3 was generated by toluene. This aromatic hydrocarbon must have been formed by isomerization of tropilidine produced, presumably, by detachment of the N—CH_3 bridge from the molecule of tropidine (or tropanol). Isomerization of tropilidene to toluene has been the subject of several recent publications (7–12). Under the present experimental conditions hydrogen transfer reactions may also take place. Thus peaks No. 1 and 2 were assigned to pyrrolidine and piperidine. Peaks No. 6 and 7 appeared to be due to partially dehydrogenated piperidines. Peaks at the same retention times were observed along with the peaks of pyridine and unchanged piperidine, when the latter was passed through the reactor under the same experimental conditions. Highly volatile products, *e.g.*, methane, ethylene, methylamine, *etc.*, were presumably eluted within the first 2 min., and their peaks were masked by those generated by ethanol, the solvent employed for injecting the samples.

Atropine and Homatropine—Atropine and homatropine are esters of tropanol with tropic and mandelic acids. Pyrolysis of these substances would be expected to yield tropidine and the corresponding acids, which products would undergo further degradation to simpler molecules. Thus the reaction chromatograms of these alkaloids (Fig. 1,B and C) exhibited all the peaks generated by tropanol. In addition atropine showed a peak (No. 8) which, according to ultraviolet spectral analysis, may be assigned to styrene. This product was also obtained when tropic acid was passed through the reactor under the same experimental

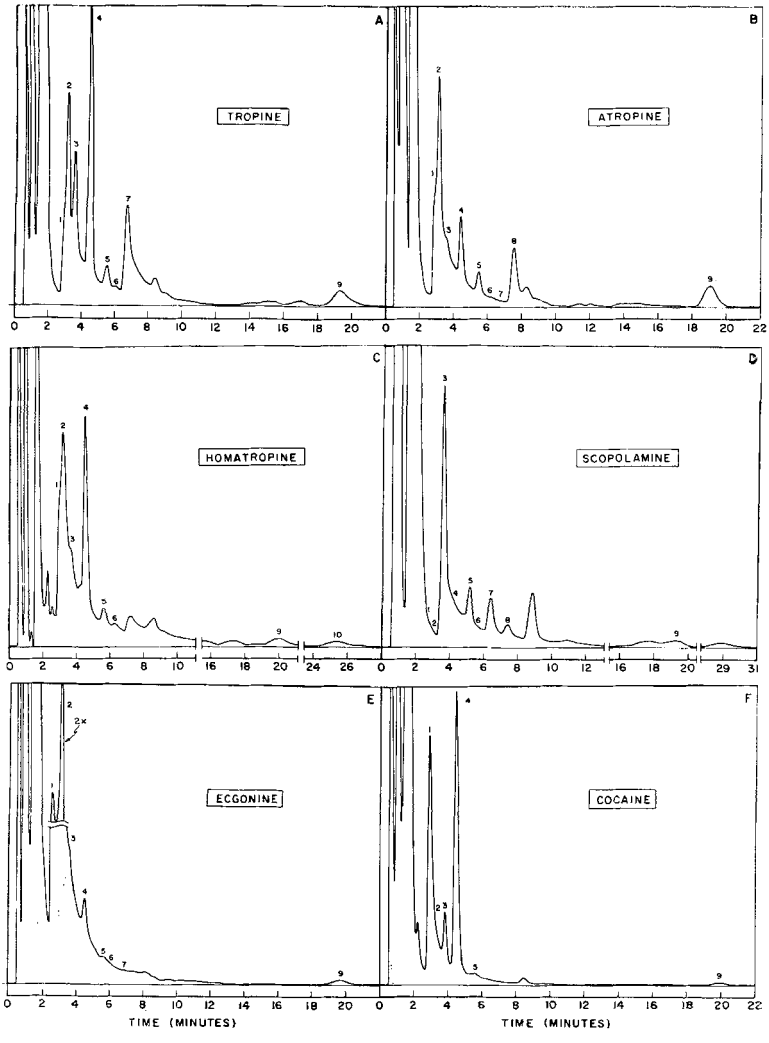
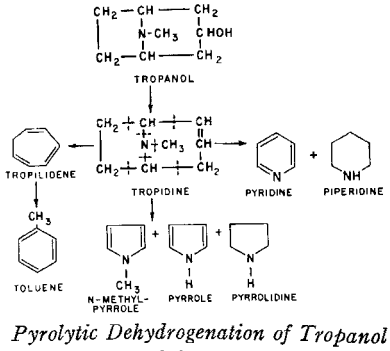


Fig. 1—Reaction chromatograms of tropanol and tropane alkaloids. (See Table II for assignment of peaks.)



Pyrolytic Dehydrogenation of Tropanol
Scheme I

conditions. The reaction chromatogram of homatropine exhibited a peak due to benzaldehyde, formed by degradation of mandelic acid moiety in the alkaloid. This was confirmed by injecting mandelic acid in the reactor, when benzaldehyde was observed to be the main product of reaction.

$$C_6H_5CH(COOH)CH_2OH \rightarrow C_6H_5CH{=}CH_2$$
$$C_6H_5CH(OH)COOH \rightarrow C_6H_5CHO$$

TABLE II—ASSIGNMENT OF PEAKS IN REACTION CHROMATOGRAMS

Peak No.[a]	Relative Retention Time[b]	Assignment
1	0.64	Pyrrolidine
2	0.71	Piperidine
3	0.82	Toluene
4	1.00	*N*-Methylpyrrole
5	1.25	Pyridine
6	1.38	Partially dehydrogenated piperidines
7	1.52	
8	1.68	Styrene
9	4.55	Pyrrole
10	5.83	Benzaldehyde

[a] See Fig. 1. [b] Reference standard: *N*-methylpyrrole.

Scopolamine—Scopolamine is an epoxy-atropine. On pyrolytic dehydrogenation it yielded products many of which were the same as those produced by atropine (see Fig. 1,D). However, the peaks due to *N*-methylpyrrole, pyrrole, and pyrrolidine

were comparatively smaller presumably due to further oxidative degradation of the five-membered heterocyclic ring carrying the epoxy group.

Ecgonine and Cocaine—Ecgonine is a carboxy derivative of tropanol, while cocaine is a carbomethoxy derivative of tropanyl benzoate. Although these compounds would be expected to yield the same products of degradation, the yield of six-membered heterocyclic compounds was in fact, very poor (Fig. 1,E and F). Presumably these compounds do not decarboxylate easily and are retained by the basic support.

CONCLUSIONS

The experimental data reported above demonstrate that the described technique should prove of value in establishing the identity of tropane alkaloids as well as in recognizing the tropane carbon skeleton in alkaloids of unknown structure.

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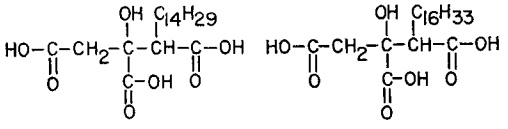
Pharmacologic Study of Norcaperatic and Agaricic Acids

By RICHARD A. CARRANO* and MARVIN H. MALONE

In rats norcaperatic acid produced delayed onset, dose-related effects of mydriasis, skeletal muscle weakness, and central nervous system depression similar to those induced by its homolog, agaricic acid. Agaricic acid was a nonspecific potentiator of furtrethonium in isolated rat jejunum. Both acids produced a slow onset of leiomyotonic effects on isolated guinea pig ileum. This effect could be blocked by papaverine but was not affected by high concentrations of atropine, pyribenzamine, or by a depolarized muscle. Tonic activity could also be produced in a K^+ deficient muscle. Both norcaperatic acid and agaricic acid were competitive inhibitors of the enzyme aconitase with norcaperatic acid being slightly more potent. The potentially toxic properties of *Cantharellus floccosus* due to the presence of norcaperatic acid may be related to the structural chemical relationships of this substance to citric acid.

CANTHARELLUS FLOCCOSUS Schw. has been reported to cause serious delayed gastrointestinal disturbances in individuals (1). Miyata *et al.* (2) isolated and characterized the active principle as norcaperatic or α-tetradecylcitric acid ($C_{20}H_{36}O_7$). Agaricic acid ($C_{22}H_{40}O_7$), an isolate from the mushroom *Polyporus officinalis* Fr., is a homolog of norcaperatic acid and has been included in this study (I and II).



NORCAPERATIC ACID I

AGARICIC ACID II

The isolation of norcaperatic acid from *C. floccosus* marked its first reported occurrence in nature, although agaricic acid had been isolated and studied by various investigators and classified as an anhydrotic and parasympatholytic agent (3–5). The literature reports do not satisfactorily settle whether the mechanism of action on smooth muscle is musculotropic or neurotropic. This study hoped to clarify this problem and to study the possible mechanisms by which these agents could induce mushroom poisoning.

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