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SHORT COMMUNICATION

COMPOSITION STUDIES ON TOBACCO—XXVIII.

2,3,6-TRIMETHYL-1,4-NAPHTHOQUINONE IN CIGARETTE SMOKE

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Abstract—2,3,6-Trimethyl-1,4-naphthoquinone was isolated from the nitromethane-soluble, neutral fraction of cigarette smoke condensate and identified by spectral (i.r., mass and NMR) and gas chromatographic characteristics. This is the first report of the occurrence of quinones in tobacco smoke.

The nitromethane-soluble neutral fraction of cigarette smoke condensate has cocarcinogenic activity when tested in animals[1] and the compounds responsible for this effect are unidentified. Recent work on this fraction has resulted in the isolation of myristicin,[2] benzyl esters,[3] cinnamonitrile,[3] and secondary aromatic amines.[4] The present report describes the identification of a quinone in this fraction. The presence of this class of compounds in smoke has not been known previously. Citation of the presence of 1,4-benzoquinone in cigarette smoke is erroneous.[5] Actually, several reports[6–10] on the isolation of hydroquinone from smoke have appeared including one[6] in which the authors suggested that the compound was present in smoke in the quinone form but presented no experimental evidence on this point.

Smoke condensate was fractionated by solvent partition and the nitromethane-soluble, neutral subfraction was chromatographed successively on silicic acid and neutral alumina, using a flowing elution technique. A fraction eluted with benzene–light petroleum from alumina gave at least thirty peaks on gas chromatographic separation. Multiple injections and collections of one peak yielded about 1·8 mg of a yellow crystalline solid. The i.r. spectrum of the unknown showed very strong absorption at 6·05 μ, indicative of a highly conjugated carbonyl. A strong doublet at 6·20 and 6·28 μ suggested the presence of aromaticity but the intensity of possible aromatic bands at 11·80 and 14·00 μ appeared somewhat weak

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[1] F. G. Bock. Unpublished data.

[2] I. Schmeltz, R. L. Stedman, J. S. Ard and W. J. Chamberlain, *Science* **151**, 96 (1966).

[3] I. Schmeltz, C. J. Dooley, R. L. Stedman and W. J. Chamberlain, *Phytochem.* **6**, 33 (1967).

[4] R. L. Miller, L. Lakritz, C. J. Dooley and R. L. Stedman, *Tobacco Sci.* **11**, 35 (1967).

[5] H. R. Bentley and E. G. N. Berry, *The Constituents of Tobacco Smoke: An Annotated Bibliography*, Research Paper No. 3, Tobacco Manufacturers Standing Committee, London, England (1959).

[6] E. J. Bonnet and S. Neukomm, *Helv. Chim. Acta* **40**, 717 (1957).

[7] K. Herrmann, *Beitr. Tabakforsch.* **2**, 159 (1964).

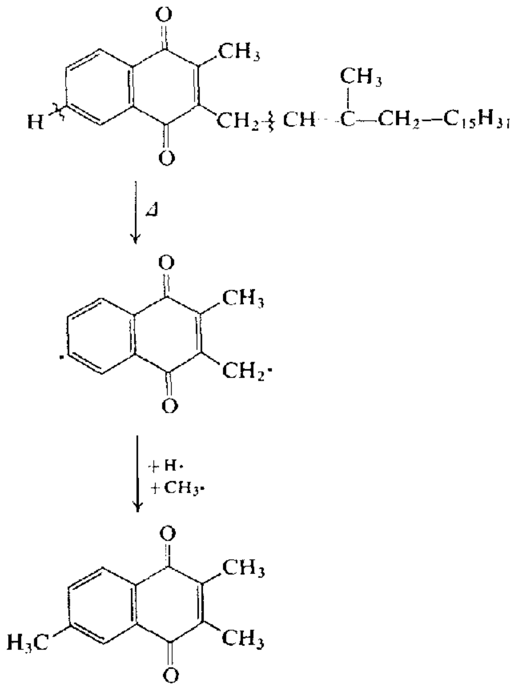
[8] R. A. W. Johnstone and R. L. Plimmer, *Chem. Rev.* **59**, 885 (1959).

[9] G. A. L. Smith and D. A. King, *Chem. & Ind.* **540** (1964).

[10] A. Testa, P. Testa and J. Cuzin, *Serv. Explot. Ind. Tab. Allumette Ann Sect.* **1**, 87 (1965); *Tobacco Abstr.* 909 (1966).

to confirm an aromatic structure. The band at 11·80 μ was just beyond the range of *cis*-unsaturation, which, if present, might enhance the doublet in the presence of aromaticity and account for the discrepancy in absorptive intensities in the 6·20–6·30 and 11·00–14·00 μ regions. The u.v. spectrum showed a doublet (s, 250 and 255 nm) and two single peaks (m, 274 nm; w, 335 nm) which were suggestive of K, B and R bands. These data indicated the unknown to be a possible highly substituted quinone.

The mass spectrum of the unknown gave a molecular peak of 200 and fragments of 185 (loss of $—CH_3$), 172 (loss of $>C{=}O$) and 157 (loss of $—CH_3$ and $>C{=}O$). A fragment of mass 82 was also obtained and was attributable to $—C(CH_3){=}C(CH_3)\overset{\overset{O}{\|}}{C}—$; quinones are known to give a three-carbon scission in this manner.[11] Since tetrasubstituted olefins absorb in the 6·0–6·3 μ region ($C{=}C$ stretching) but not in the 10·0–15·0 μ region ($C—H$ stretching and deformation), the above discrepancy in absorptions of the unknown in the two ranges was resolved. Apparently, the $C{=}C$ stretching of the olefinic moiety occurs with and enhances the aromatic doublet. No tropylium ion was observed. The NMR spectrum showed two sets of methyl protons (τ7·9, 6H, $CH_3\overset{|}{C}{=}\overset{|}{C}—$ and τ7·55, 3H, $Ar—CH_3$) and aromatic protons (τ2·0–2·6, 3H). The small sample size gave high noise levels and made evaluations of the multiplets in the aromatic proton region impossible.



This evidence showed the presence of a probable substituted 2,3-dimethyl-1,4-naphthoquinone structure containing a methyl group in the aromatic ring. The two possible isomers

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[11] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Interpretation of Mass Spectra of Organic Compounds*, p. 203. Holden-Day, San Francisco (1964).

were synthesized by chromic acid oxidation of the corresponding trimethylnaphthalenes.[12] The synthetic 2,3,6-trimethyl-1,4-naphthoquinone had i.r., u.v. and mass spectra identical to that of the unknown. Gas chromatography of the isolated compound and the synthetic compound gave a single peak on an SE-30 column.

The origin of the compound in smoke is of interest. Two possible mechanisms are evident: vapor phase oxidation of alkylnaphthalenes known to be in smoke, and partial thermal degradation of quinones present in leaf. Since 2,3,6-trimethylnaphthalene is not a smoke constituent, the first possibility is less favorable since rearrangements of methyl groups from known trimethylnaphthalenes are required. Reported quinones of tobacco leaf are anthraquinone,[13] 2-methyl-3-phytyl-1,4-naphthoquinone[14] (Vitamin K_1) and two possible analogs of plastoquinone[15] (2,3-dimethyl-5-solanesyl-1,4-benzoquinone) which have not been characterized. It might arise from Vitamin K_1 as shown in Fig. 1.

Apparently, other methyl-substituted quinones of this type occur in smoke, but none has been isolated and identified thus far. However, 3-phenylpropionitrile ("dihydrocinnamonitrile") was separated from the same fraction as the identified quinone. The occurrence of this nitrile in cigar smoke was reported recently.[16]

EXPERIMENTAL

Fractionation of Smoke Condensate

Smoke condensate (1 kg) obtained by mechanical smoking of approximately 50,000 domestic, 85 mm, nonfilter cigarettes was fractionated as described.[3] The nitromethane-soluble neutral fraction (36·7 g) was chromatographed on methanol washed, activated (150° for 17 hr) silicic acid (800 g in a 64 cm × 59 mm column). The column was eluted successively with 25 per cent benzene in light petroleum (5·5 l.), benzene (3·95 l.), 25 per cent diethyl ether in benzene (7·05 l.) (A) and diethyl ether (3·1 l.). The material eluted with A (26·5 g) was then fractionated on neutral alumina (400 g in a 50 cm × 38 mm column) activated at 170° for 18 hr. The first fraction (3·4 g) eluted with 30 per cent benzene–light petroleum was separated by gas chromatography (elution time, 41 min) under the following conditions: 3 m × 6 mm column of 20 per cent dimethyldichlorosilane treated SE-30 on Chromosorb D; detector, 300°; injector, 300°; flow rate (helium), 10 ml/min; and temperature programmed at 4°/min from 70° to 145° and then 2°/min from 145° to 280°.

Preparation of 2,3,5- *and* 2,3,6-*trimethyl*-1-1,4-*naphthoquinone*

The hydrocarbon (2 g) was dissolved in glacial acetic acid (70 ml). To this solution was added a solution of CrO_3 (4·3 g) in 26 ml of acetic acid and 7·8 ml of water. The temperature was maintained between 20 to 30° during the addition by means of an ice bath. The mixture was allowed to stand for 72 hr and was then diluted with 300 ml of water. After standing another 4 hr the solution was filtered. The crude quinones were purified by crystallization from ethyl alcohol and gas chromatography.

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[15] D. R. Threlfall, W. T. Griffiths and T. W. Goodwin, *Biochim. Biophys. Acta* **102**, 614 (1965).

[16] S. Osman and J. Barson, *Chem. & Ind.* **699** (1966).