Studies on the Constituents of Hemp Plant (*Cannabis sativa* L.)

Part I. Volatile Phenol Fraction

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Examination of the volatile compounds from Japan hemp plant shows that the phenol fraction consists mainly of eugenol with small amounts of guajacol. Two unknown carbonyl compounds are separated as their 2,4-dinitrophenylhydrazones by the silicic acid-celite column chromatography from the phenol fraction.

INTRODUCTION

As the volatile constituents of hemp resin, a terpene b.p. 165~175°C, possibly myrcene, and a sesquiterpene were reported by Wood, Spivey, and Easterfield¹⁾. In 1942, Simonsen and Todd²⁾ examined the essential oil from Egyptian hashish of uncertain age, and showed that the "lower-boiling terpene fraction" consists mainly of *p*-cymene with small amounts of 1-methyl-4-isopropenyl-benzene and unidentified optically active material. From the "higher-boiling fraction" of the oil, humulene (α -caryophyllene) was isolated.

In the course of our investigations on hemp plant constituents, the authors examined the volatile phenol fraction and identified eugenol and a trace of guajacol by paper chromatography. After purification of 3,5-dinitrobenzoate derived from the phenol fraction by silicic acid-celite column chromatography, mixed melting points, elemental analysis and infrared spectra proved it as eugenol 3,5-dinitrobenzoate. Eugenol has never been isolated from Moraceae family.

From the aqueous solution of 20% sodium bisulfite which was used to separate the carbonyls from the crude phenol fraction, two unidentified

2,4-dinitrophenylhydrazones, m.p. 210°C and 105~107°C were obtained.

EXPERIMENTAL

Sample:

Minamioshihara No. 1 variety of the domestic hemp, raised in the experimental field of Hokkaido University during the season of 1958 was used in this study. It was cut in August just before efflorescence. About 100 kg of hemp leaves and tops were gathered immediately and extracted with petroleum ether (b.p. 30~50°C) at the room temperature. Extraction was repeated and the total number of 210 l of petroleum ether was used. Most of the solvent was recovered under 70°C of water bath and 22.2g of the solids was separated by centrifuge.

The supernatant, about 2 l, was shaken with 5% sodium bicarbonate. After combining the petroleum ether layer with the washings of the precipitate which was formed by the shaking with bicarbonate, 5 l of the solution was shaken with aqueous 5% sodium hydroxide solution. The aqueous extract, acidified with sulfuric acid and saturated with sodium chloride, was shaken with ethyl ether repeatedly and thus crude phenols were obtained. Yield 4.9g, red oil. The crude phenols were steam-distilled, and the distillate of an amount of 2.5 l was saturated with sodium chloride and extracted with ether repeatedly. The volatile phenol fraction, red oil, was obtained from the ether solution. Yield 1.4g.

Detection of Phenols by Paper Chromatography:

Detection of phenols in the volatile phenol fraction was carried out by paper chromatography, preparing

1) T. B. Wood, W. T. N. Spivey and T. H. Easterfield, *J. Chem. Soc.*, **69**, 539 (1896).

2) J. L. Simonsen and A. R. Todd, *J. Chem. Soc.*, **1942**, 188.

their oxyazocompounds according to the methods of Hossfeld et al.^{3,4)}. Two drops of the crude phenol fraction was dissolved in 0.1 ml of aqueous 8% sodium hydroxide. Three drops of diazotized sulfanilic acid were added in this solution. After being acidified with an excess of concentrated hydrochloric acid, the reaction mixture was extracted once with benzene. The aqueous solution was diluted with alcohol and spotted on the position at 4 cm from the below end of the filter paper which had been sprayed with aqueous 4% sodium carbonate and dried. Water saturated *n*-butanol, a mixture of *n*-butanol add 28% ammonia water (4:1, v/v) or *sec*-butanol saturated with aqueous 2% sodium carbonate were used for irrigating solvent and developed upward at the room temperature. Eugenol and traces of guajacol and few other phenolic compounds were detected in each solvent system.

Isolation and Identification of Eugenol 3,5-Dinitrobenzoate:

The crude phenol fraction was dissolved in 50 ml of ether and shaken with an aqueous solution of 20% sodium bisulfite to separate the carbonyls. After removal of the carbonyl compounds and being dried, about 1 g of red oil was dissolved in 3 ml of anhydrous pyridine and 0.5 g of 3,5-dinitrobenzoyl chloride was added. The mixture was warmed over a low flame for a minute and poured into 10 ml of water. The light brown precipitate was separated and stirred thoroughly with 20 ml of 2% sodium bicarbonate solution, and recrystallized from ethyl alcohol. One hundred and sixty mg of slightly colored needles m.p. 130°C was obtained.

The 3,5-dinitrobenzoate was dissolved in a small amount of benzene and added on the top of the column consisting with silicic acid and celite mixed at the rate of 2:1 in weight. The column, 10 cm in its height, was eluted with a mixture of petroleum ether and ethyl ether (5% by volume). Total eluate from the column was 800 ml. The solvent was evaporated and the residue was recrystallized from ethyl alcohol. White crystals, 57 mg, m.p. 130~131°C. An additional crops of 41 mg, m.p. 130~131°C, was obtained from the filtrate. *Anal.* Found: C, 57.0; H, 4.0; N, 7.58. Calcd. for C₁₇H₁₄O₇N₂: C, 57.0; H, 3.9; N, 7.82.

The infrared spectrum of this crystal was identical with 3,5-dinitrobenzoate of the authentic eugenol. The mixed melting point of these derivatives did not depress.

Carbonyls in Crude Phenol Fraction:

3) R. L. Hossfeld, *J. Am. Chem. Soc.*, **73**, 852 (1951).

4) Wen-Hua Chang, R. L. Hossfeld and Wm. M. Sandstrom, *J. Am. Chem. Soc.*, **74**, 5766 (1952).

The aqueous 20% sodium bisulfite solution which was used to separate the carbonyls from the volatile phenol fraction was washed with ether, added with 6N sulfuric acid and extracted repeatedly with ether. After distilling off the ether, about 400 mg of red oil was obtained. This oil was poured into the solution consisting of 0.5 g 2,4-dinitrophenylhydrazine, 2 ml concentrated sulfuric acid, 3 ml water and 40 ml ethyl alcohol. After a few days, a separated reddish precipitate of 2,4-dinitrophenylhydrazone, 160 mg, was filtered off and dried over P₂O₅.

Column Chromatographic Separation of 2,4-dinitrophenylhydrazones:

A mixture of silicic acid and celite, at the rate of 2:1 in weight, was added with petroleum ether into a chromatographic glass tube to build a column (27×250 mm). A chloroform solution of 20 mg 2,4-dinitrophenylhydrazones was poured onto the column. After absorption of the chloroform solution, 200 ml of petroleum ether containing ethyl ether (2% by volume) was added on the column. As separation of the bands occurred, the ethyl ether content of the mixture solvent was increased to 5% and 10% (by volume) and eluted 300 ml and 750 ml respectively. After the first yellow zone (Band No. 1) had been eluted out, 4 bands (No. 2~5) were extruded, sectioned and eluted with ethyl ether. The result obtained with three repetition are shown in Table I.

TABLE I. CHROMATOGRAPHIC SEPARATION OF 2,4-DINITROPHENYLHYDRAZONES OBTAINED FROM THE VOLATILE PHENOL FRACTION OF HEMP PLANT

Band No.	Color of Band	Yield (mg)
1	Yellow	37
2	Orange	20
3	Orange	
4	Yellow	6
5	Orange brown	4

Band No. 1:

Two successive recrystallization from a mixture of petroleum ether and ethyl ether gave yellow crystals, m.p. 105~107°C, 20 mg. The infrared spectrum of this crystal, shown in Fig. 1, was compared with those of authentic 2,4-dinitrophenylhydrazones of *n*-heptylic aldehyde (m.p. 105~106°C) and *n*-valeric aldehyde (m.p. 107~108°C), however, did not agree with each one of them.

Band No. 2:

The 2,4-dinitrophenylhydrazone, remained in the flask after removing of the solvent used to elute the orange band (No. 2), was recrystallized from a mixture of ethyl

FIG. 1. Infrared Absorption Spectrum of Unknown 2,4-Dinitrophenylhydrazone (m.p. 105~107°C, Band No. 1). Obtained from Hemp Plant (Nujol).

FIG. 2. Infrared Absorption Spectrum of Unknown 2,4-Dinitrophenylhydrazone (m.p. 210°C, Band No. 2). Obtained from Hemp Plant (Nujol).

ether and chloroform and the melting point was raised to 210°C. The infrared spectrum of this red crystal, shown in Fig. 2, did not agree with authentic 2,4-dinitrophenylhydrazone of *o*-hydroxyacetophenone (m.p. 210~213°C) or 5-methyl furfural (m.p. 213~214°C) prepared from sucrose according to the method of Scott and Johnson⁵⁾.

We could not identify any component of the carbonyls

because of the lack of sufficient amount for further investigation.

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5) E.W. Scott and J.R. Johnson, *J. Am. Chem. Soc.*, **54**, 2549 (1932).