PEPPER ANALYSIS

Composition of Volatile Oil of Black Pepper, *Piper nigrum*

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The composition of the essential oil of black pepper, *Piper nigrum*, is described. The characteristic odor of this oil is due to hitherto unreported small amounts of oxygenated terpenes, of which piperonal, dihydrocarveol, caryophyllene oxide, cryptone, and an alcohol $C_{10}H_{18}O$ were isolated and identified. The results are for use in the formation of an acceptable synthetic pepper to be used in case of a national emergency.

THE VOLATILE OIL COMPOSITION of black pepper, *Piper nigrum*, has been studied at intervals by several investigators for more than a century. Dumas (*7*), Suberiran and Capitaine (*25*), and Eberhardt (*9*) recognized that the oil is almost entirely free of oxygenated compounds. Schimmel and Co. (*22*) and Schreiner and Kremers (*23*) showed the presence of 1-α-phellandrene, dipentene, and caryophyllene in the oil, but little else was known about its composition.

The material used for the investigation herein reported was an oil obtained by steam distillation from freshly ground Malabar peppercorns harvested in 1950. Preliminary experiments had shown that solvent extraction of ground pepper, followed by removal of solvent and steam distillation of the extract to recover the volatile oil, offered no advantange over direct steam distillation (*12*).

The steam-distilled pepper oil was washed neutral with aqueous sodium bicarbonate solution, dried, and fractionated into monoterpenes, oxygenated monoterpenes, and sesquiterpenes. The sodium bicarbonate wash yielded phenylacetic acid after acidification. The practical difficulties in separating the oxygenated components of pepper oil from hydrocarbons by fractional distillation led to the study of their chromatographic separation. Pepper oil from which the monoterpenes had been removed by distillation was chromatographed on a column of aluminum oxide. The ligroin eluate, containing the remaining hydrocarbons, was distilled and yielded in the last traces of the caryophyllene fraction a blue liquid hydrocarbon, apparently an azulene, in minute amounts. Its ultraviolet spectrum (max. λ 254 and λ 264 mμ) did not correspond to any of the common azulenes.

A crystalline compound, present in the early eluates, was found to be epoxydihydrocaryophyllene. The presence of this compound in steam-distilled pepper oil is interesting in view of the question of the origin of caryophyllene and its relationship to the oxide in essential oils. Treibs (*26*) showed that steam-distilled clove oil contains small amounts of epoxydihydrocaryophyllene and suggested that it is formed from caryophyllene on oxidation. More recently, Naves (*17*) examined solvent-extracted clove oil and found it contained no caryophyllene. Naves found that the neutral nonvolatile portion of the clove extract yielded caryophyllene after steam distillation. Black pepper oleoresin obtained by the extraction of ground pepper with benzene was therefore subjected to distillation under high vacuum at low temperatures. No caryophyllene was obtained in this manner, although small amounts of caryophyllene added to the oleoresin were readily removed by distillation under the same experimental conditions. This suggests that black pepper spice, like cloves, does not contain caryophyllene as a primary component.

The basic fraction of pepper oil was found to contain piperidine, identified as *N*-phenyl-*N'N'*-pentamethylenethiourea. Its presence had been reported

Table I. Components Isolated from Pepper Oil

Compound	Formula	B.P. or M.P., °C.	Estimated Oil, %	Characterization	M.P., °C.	Analysis					
						Calculated			Found		
						C	H	N	C	H	N
				Nitrolpiperidide (*10, 28, 24*)	117	72.00	10.40	11.20	72.31	10.43	11.43
α-Pinene	$C_{10}H_{16}$	157–161	14	Myrtenal semicarbazone (*8*)	206	63.77	8.21	20.29	63.93	8.02	20.30
β-Pinene	$C_{10}H_{16}$	164.5–168	23	Nopinic acid (*1, 14*)	126–127	65.18	8.75	...	65.41	8.41	...
1-α-Phellandrene	$C_{10}H_{16}$	175–178.5	7	Maleic anhydride adduct (*6*)	125	71.80	7.69	...	71.72	7.67	...
dl-Limonene	$C_{10}H_{16}$	178.5–181	25	Tetrabromide (*3, 11*)	124–125	26.34	3.53	...	26.37	3.42	...
β-Caryophyllene	$C_{15}H_{24}$	126.5/20 mm.	19	Phenylurethane of caryophyllene alcohol (*2*)	135–136	77.38	9.15	...	77.64	9.13	...
Epoxydihydrocaryophyllene	$C_{15}H_{24}O$	m.p. 61–62	0.1	Mixed melt with authentic specimen	...	81.80	10.93	...	81.88	10.67	...
Phenylacetic acid	$C_8H_8O_2$	m.p. 76.5–77	0.2	Mixed melt with authentic specimen	...	70.59	5.92	...	70.81	6.04	...
Dihydrocarveol	$C_{10}H_{18}O$	108–124/20 mm.	2	3,5-Dinitrobenzoate (*13*)	120.5–121.5	58.61	5.80	8.05	58.72	5.75	8.34
Piperonal	$C_8H_6O_3$	108–124/20 mm.	0.5	Semicarbazone (*29*)	218–219	52.17	4.38	20.28	52.53	4.16	19.96
Cryptone	$C_9H_{14}O$	108–124/20 mm.	0.1	Semicarbazone (*5*)	185	61.54	8.71	...	61.39	8.79	...
Piperidine	$C_5H_{11}N$	106	0.1	*N*-Phenyl-*N'*,*N'*-pentanethylene thiourea (*15*)	99	65.41	7.31	12.72	65.54	6.99	12.92
(Alcohol)	$C_{10}H_{18}O$	103–105/13 mm.	0.1	3,5-Dinitrobenzoate	117–117.5	58.62	5.78	8.04	58.54	5.74	8.28
(Alcohol)	$C_{15}H_{26}O$	100[a]/1 mm.	0.1	...	...	81.08	11.71	...	81.19	11.47	...
(Alcohol)	$C_{15}H_{24}O$	110[a]/1 mm.	0.1	...	...	81.81	10.90	...	81.83	11.08	...
Citronellol (?)	...	...	0.1	Semicarbazone of pyruvic acid ester (*4*)	...	...	...	...	...	...	...
	$C_{15}H_{32}O_4$	m.p. 97	...	...	...	64.81	11.67	...	64.45	11.91	...
	$C_9H_{16}O_2$	m.p. 161	0.1	...	...	69.23	10.26	...	69.20	10.32	...
	$C_{10}H_{10}O_2$	m.p. 234–235	0.1	...	...	74.07	6.17	...	73.64	5.87	...
Higher boiling components	...	...	4.0	...	...	...	...	...	...	...	...
Residue	...	...	2.3	...	...	...	...	...	...	...	...

[a] Bath temperatures.

more than 50 years ago by Johnstone (*16*), but later denied by Pictet (*18, 19*) who isolated α-methylpyrroline from the spice. α-Methylpyrroline could not be isolated in this study by following the procedure of Pictet.

By means of fractional distillation, chromatographic separation, and chemical procedures, the following compounds were isolated: α-pinene, β-pinene, *dl*-limonene, 1-α-phellandrene, caryophyllene, dihydrocarveol, epoxydihydrocaryophyllene, piperonal, cryptone, phenylacetic acid, piperidine, an alcohol, $C_{10}H_{18}O$, and oxygenated compounds of unknown composition with the formulas $C_9H_{16}O_2$, $C_{10}H_{10}O_2$, $C_{15}H_{24}O$, $C_{15}H_{26}O$, and $C_{15}H_{32}O_4$.

About 5% of the volatile oil consists of oxygenated compounds. Some of them could not be studied further because of the extremely small amounts isolated. The presence of citronellol was indicated, but the quantity of the derivative, as the semicarbazone of the pyruvic ester, was too small for final purification.

Analytical data suggest that listed unknown components are monoterpene glycols, sesquiterpene alcohols, and glycols.

Experimental

Raw Material. The 1000-pound lot of black pepper, *Piper nigrum*, used in this investigation was harvested in January 1950, in the province of Malabar, India (purchased from McCormick and Co., Inc., Baltimore, Md.), and cured by sun-drying. Volatile and fixed oils, as determined by a modification of the standard Richardson ether extraction gravimetric method (*27*), were: fixed oils, 5.9%; volatile oils, 3.2%.

Separation of Components. Peppercorns in 20-pound batches were ground together with solid carbon dioxide in a Fitzpatrick mill. The resulting powder was slurried in 35 liters of water and steam-distilled with mechanical stirring. The distillate was extracted with ether, and the ether layer was washed with dilute aqueous solutions of boric acid and sodium bicarbonate. The solvent was removed by distillation and the residual oil showed: n_D^{25} = 1.4730, d = 0.8500, a positive Schiff test, and negative tests for nitrogen, sulfur, and esters.

The washed pepper oil was partially separated from the monoterpene hydrocarbons by vacuum distillation through a Stedman packed column. The monoterpene hydrocarbons were fractionated further through a Naragon-Lewis concentric tube column at atmospheric pressure.

The still pot residue was adsorbed on aluminum oxide (Alcoa F-20) to effect a crude separation of the remaining hydrocarbons from oxygenated compounds. Petroleum ether was used to elute the former and methanol, the latter. The hydrocarbon portion was redistilled (at 135° to 145° C. at 24 mm.), and the blue residue was further purified chromatographically. The methanol eluates were combined, the solvent was removed by distillation, and the residual oil was dissolved in ether. The ether solution was washed with 5% sodium hydroxide, but no phenolic components could be isolated from the washings. The alkali-washed neutral oil was treated with Girard T reagent in the manner recommended by Sandulesco (*20*). The yield of carbonyl compounds recovered from the water-soluble derivative by acid hydrolysis was 0.6% of the total volatile oil.

The noncarbonyl components of the

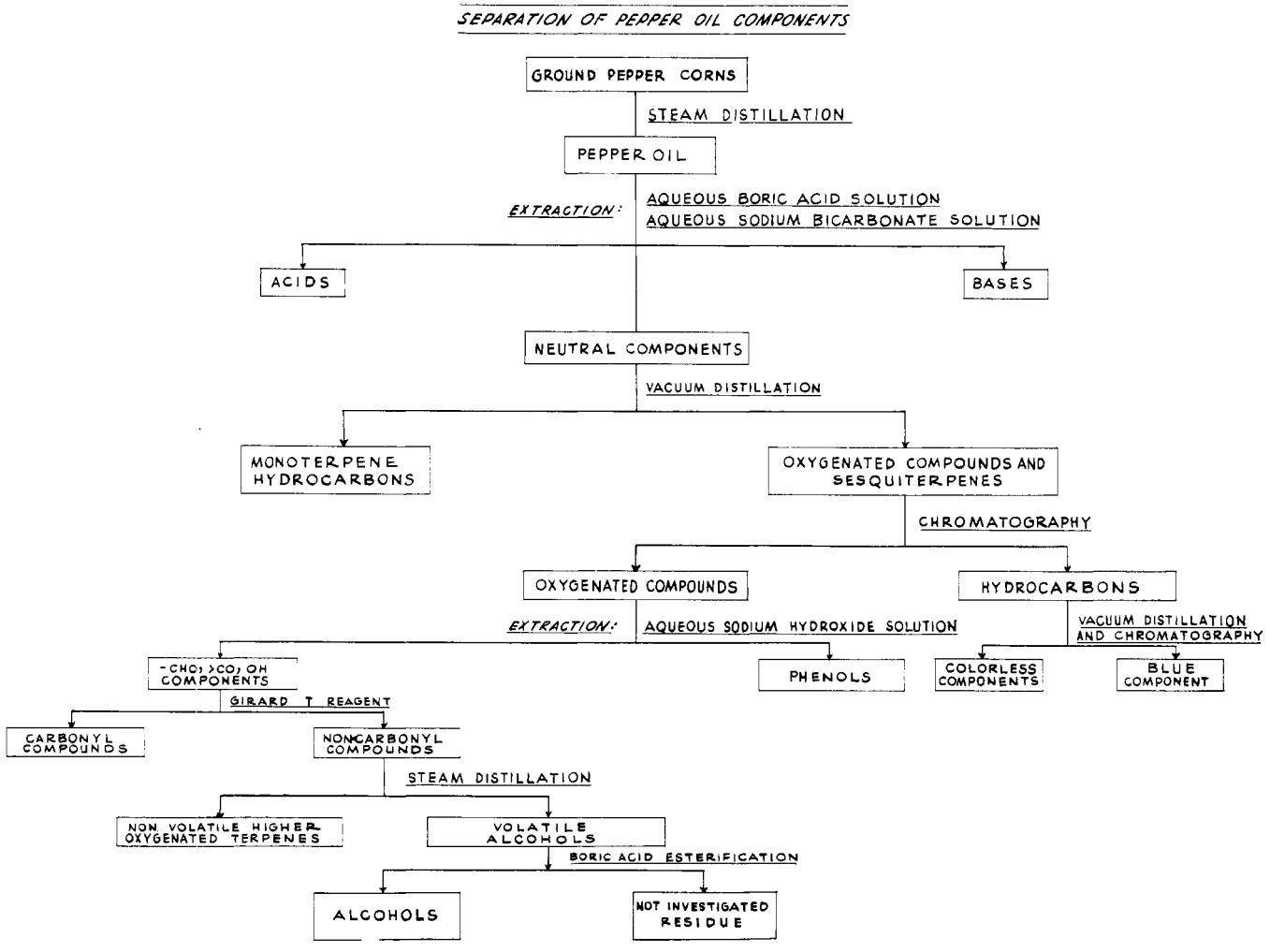

Figure 1. Separation of pepper oil components

neutral oil were steam distilled, and the components that were not readily steam volatile (0.8% of the total oil) were extracted from the still pot residue with ether and distilled in vacuo at 125° to 145° C. at 3 mm. This fraction was purified further by chromatography on aluminum oxide and refractionated. The steam-volatile components were extracted with ether from the distillate. The alcohols were separated, after removal of the solvent by esterification with boric acid, following the procedure of Scattergood, Miller, and Gammon (*21*).

The separation of pepper oil components is summarized as a flow sheet in Figure 1.

Identification of Components. Compounds isolated from pepper oil and pertinent characterization data are given in Table I.

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