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STUDIES ON THE ESSENTIAL OILS OF THE PAKISTANI SPECIES OF THE FAMILY UMBELLIFERAE

Part V. Carum Roxburghianum (Bal-Ajowan) Seed Oil

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Abstract. The chemical composition of the essential oil of the fresh and mature seed of *Carum roxburghianum* has been determined for the first time and compared with that of the one-year-old premature seed commonly sold in the market. The respective oils obtained in 3.8% and 2.5% yields are composed of α-pinene (0.66, 0.42%), myrcene (0.56, 0.88%), Δ^3-carene (0.33, 0.60%), limonene (15.06, 20.77%), γ-terpinene (1.92, 2.56%), *p*-cymene (1.92, 0.77%), *l*-cadinene (23.28, 29.0%), unidentified esters (5.60, 5.0%), fenchone (1.80, 2.0%), an acid (25.0, 18.50%), thymol (2.0, 1.6%), linalool (1.3, 1.2%), *dl*-piperitone (1.2, 1.3%), seselin (13.1, 10.2%), α-terpineol (1.4, 1.2%), piperitol (traces, traces), bergaptene (0.5, 0.1%) and *iso*-pimpinellin (0.8, 0.5%). The acid appears to be new on the basis of its IR, NMR and chemical studies. The amount of coumarins in the oils varies from 3.1-3.4%. The composition of the oil varies depending on the age and the degree of ripeness of the seed.

Carum roxburghianum vegetates commonly on the plains of the Punjab province, particularly in the Gujranwala district. Together with *Trachyspermum ammi* (Ajowan), *Apium graveolens* (Celery) and *Hyoscyamum* (Hanbane), it is used for various stomach ailments. Its seeds have since long been used as medicine for hiccough, vomiting and pains of the bladder and as an ingredient of carminative and stimulant preparations. The water of distillation (arak) of the seed has been used as a carminative and for the prevention and cure of cholera, hysteria and spasmodic attacks.

The present studies have been carried out because firstly our surveys have shown that over 100 tons of the seed of the species are collected and brought annually to markets in Pakistan and secondly they aim at highlighting the relative status and commercial importance of the essential oil as against similar oils produced elsewhere in the world.

Experimental

Materials and Methods. The mature and fresh seed of the species was hand-collected in the Gujranwala district. The one-year-old and premature seed was purchased from the Lahore market.

The essential oil was recovered by dry steam distillation of the crushed seed.[1] About 0.2% of a diethyl ether extractable fraction was also obtained from the aqueous distillate and studied separately.

The general methods used for these studies have already been reported in our earlier communications.[1] A Beckman DB spectrophotometer, in addition to the instruments employed earlier, was used to record UV spectra.

Chromatographic Analysis of the Oil. The oils from the mature and the premature seeds were fractionated into hydrocarbons and oxygenated components using silica gel chromatography[2,3] and different systems of solvents (Table 2). Twenty fractions (40-80 ml each) were collected and the successive lots with similar behaviour by TLC were combined giving six final fractions. The fractions containing more than one components were either rechromatographed or chemically separated into individual components and their percentage amounts estimated.

The hydrocarbon and oxygenated components of the oil, recovered by column chromatography, were examined by GLC using 3 mm × 3 m copper column packed with 20% polyethylene glycol succinate (BDH grade) on celite (60-80 mesh) and also on Apiezon column of the same dimensions. The monoterpenes were resolved at 110°. Most of the oxygenated components of the oil were resolved at 175° and identified against their standard samples. The monoterpenes and sesquiterpenes were also distilled from the oil at atmospheric pressure between 170-200° and 270-280° respectively and their identification was carried out by GLC.

Results

The physico-chemical characteristics of the oil are recorded in Table 1. The percentage composition of the various constituents of the oil as determined by column chromatography followed

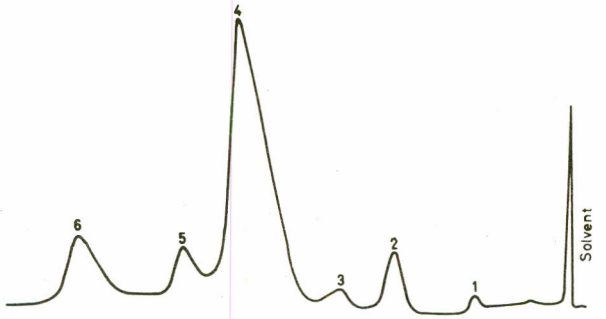

Fig. 1. Chromatogram of *Curum roxburghianum* monoterpenes run at 102°, chart speed 30 mm/min using PEGS column.

TABLE 1. PHYSICO-CHEMICAL CONSTANTS OF THE ESSENTIAL OILS OF THE *Carum roxburghianum* SEEDS.

Constants	Oil recovered from	
	Fresh mature seed	One-year-old premature seed
Yield	3.8%	2.5%
Specific gravity	0.8854^{31}	0.8648^{31}
Refractive index	1.4820^{34}	1.4870^{34}
Optical rotation	$+19° 12'^{34}$	$+35° 14'^{34}$
Acid value	3.1—3.8	1.42—1.50
Ester value	3.7—4.0	3.50—4.00

Superscripts indicate the temperature in C, at which these parameters were taken.

by GLC of the hydrocarbons fraction (Fig. 1) and their comparison with an Indian variety of similar oil have been shown in Table 2.

Discussion

The hydrocarbon fractions of the oils, as resolved by GLC, consisted of monoterpenes and sesquiterpenes in almost equal amounts. The former contained mainly limonene while the latter 1-cadinene. 1-cadinene was, however, identified by IR : S(3.4, 6.2, 7.0, 7.3 μm), M(3.3 μm), W(7.9, 8.6, 8.7, 9.1, 12.7 μm) and by the colour reaction described by Guenther.[5] Malaviya and Dutta[4] had previously detected only three monoterpenes in the essential oil of the Indian *Carum roxburghianum* seed and identified these hydrocarbons by chemical methods. However, using GLC in the present studies we have been able to show the presence of six monoterpenes in the Pakistani oil.

The oxygenated components of the oil have, nevertheless, been analysed by means of column chromatography followed by GLC of the fractions as also by fractional distillation of the oil under reduced pressure. Column chromatographic fraction 2, consisted of esters with a small amount of fenchone ; the latter being identified by GLC and IR against its standard sample. The esters have, however, to be identified as yet. The third fraction contain fenchone and linalool as identified by TLC and GLC.

Although many of the oxygenated components identified in this work have been reported by the earlier workers[4] yet the oil under our investigation seems to be different in that it contains fenchone as additional constituent and it does not contain thymoquinol and *p-iso*propyl benzoic acid as reported earlier. So far *p*-isopropyl benzoic acid is concerned its presence in the oil had been inferred by the previous workers[4] on the basis of its m.p. (116-117°). We, however, isolated a shining white crystalline compound (from fraction 5) with the same m.p. using the method employed by these workers and also by column chromatography. This compound appeared fluorescent under UV-light and was identified as seselin m.p. 117-118°, IR : S(3.4, 5.8, 6.3, 6.8, 7.3, 9.0, 12.0, 13.6 μm), M(6.1, 7.2, 7.4, 8.6, 9.3, 9.9 μm), W(7.1, 7.5, 8.1, 8.4, 10.4, 10.6, 11.0, 11.7, 12.7, 14.3, 14.8, 15.0, 15.4, 15.8 μm). With concentrated H_2SO_4 it gave umbelliferone as confirmed by UV absorption, $EtOH\lambda_{max}$ 300, 304, 326 μm (lit.[6] $EtOH\lambda_{max}$ 300, 306, 325 μm). Seselin was further identified by UV absorption ($EtOH_{max}$ 330, 293, 283, 260 μm) against its standard sample.

The mother-liquor contained mainly α—terpineol with traces of piperitol as identified by TLC and IR.

The presence or otherwise of thymoquinol, nevertheless, could not be verified, but the mother liquor left after the crystallization of the new acid and thymol (from fraction 4) indicated the existence of some phenolic matter which is as yet to be identified.

The major oxygenated component of the oil has been identified as a novel acid. The acidic fraction 4, when allowed to stand, gave rise to a crop of fine transparent crystals which were separated and recrystallised from ethanol. The acid was completely recovered from the fraction with 5% aqueous $NaHCO_3$. The portion of the fraction having no reaction with $NaHCO_3$ solution was treated with 0.5N KOH and the phenolic component, thymol (by m.p. and IR), recovered by acidification and diethyl ether extraction. The residue of the fraction contained linalool, *dl*-piperitone (by TLC and IR) and an unidentified phenol.

The m.p. (107-108°) of the novel acid is identical with the unidentified ketonic acid of the *Cerum roxburghianum* essential oil examined by Malaviya and Dutta.[4] We separated this acid from the oil by chemical and chromatographic methods and by fractional distillation of the oil under reduced pressure. We also recovered this acid by fractional distillation at atmospheric pressure according to Malaviya and Dutta[4] and obtained the same product. The NMR and elemental analysis of this acid, whether separated chemically or by column chromatography are identical. It appeared to have a molecular weight 168 via mass spectrometry. The CH and O analysis of this compound worked out to 71.08% carbon, 9.5% hydrogen and 19.35% oxygen. A formula $C_{10}H_{16}O_2$ (MW 168) would, therefore, be consistent with this analysis. The IR and NMR analysis also showed this compound as an acid.

TABLE 2. PERCENTAGE COMPOSITION OF THE ESSENTIAL OIL OF THE *Carum roxburghianum* SEEDS AND ITS COMPARISON WITH THAT OF AN OIL OF THE INDIAN ORIGIN.

Solvents used	Constituents of Pakistani oil	Fresh mature seed (%)	One year permature seed (%)	Constituents of Indian oil[4]
n-Hexane	Total hydrocarbons	44.20	55.00	
	Monoterpenes[a]	20.92	26.00	Monoterpenes
	1. α-Pinene	0.66	0.42	—
	2. Myrcene	0.56	0.88	—
	3. Δ^3-Carene	0.33	0.60	—
	4. Limonene	15.06	20.77	Limonene
	5. γ-Terpinene	1.92	2.56	γ-Terpinene
	6. *p*-Cymene	1.92	0.77	—
	—	—	—	*d*-Limonene
	Sesquiterpenes[b]	23.28	29.00	High boiling hydrocarbons
	1-Cadinene	23.28	29.00	—
	Oxygenated components[c]	55.80	45.00	Oxygenated components
2% Diethyl ether in n-hexane	High boiling esters	5.60	5.00	High boiling esters
	Fenchone	1.80	2.00	—
5—10% Diethyl ether in n-hexane	Linalool	1.30	1.20	Linalool
10—15% Diethyl ether in n-hexane	*dl*-Piperitone	1.20	1.30	*dl*-Piperitone.
	Thymol	2.00	1.60	Thymol
	New acid	25.00	18.50	Ketonic acid
	α-Terpineol	1.40	1.20	α-Terpineol
	Piperitol	Traces	Traces	—
20% Diethyl ether in n-hexane	Seselin	13.10	10.20	—
100% Diethyl ether	Bergaptene	0.50	0.10	—
	Isopimpinellin	0.80	0.50	—
5% Ethanol in diethyl ether	Unknown coumarins furocoumarins	3.10	3.40	Residue not studied
	—	—	—	*p*-Isopropyl benzoic acid
	—	—	—	Thymoquinol
	—	—	—	Cuminol (traces)

(a) Studied by gas liquid chromatography. (b) Studied by chemical method. (c) Studied by column chromatography.

The recovery of the acid by chemical method was only 10% while it was upto 25% by column chromatography and also by fractional distillation under reduced pressure followed by $NaHCO_3$ extraction. The low acid value of the oil also indicates that the acid reacts slowly with alkalies in the presence of other constituents. It has also been observed that regeneration of *dl*-piperitone from its semicarbizone derivative gave some crystalline substance as well and it was identified to be the acid mentioned above. It shows that the acid probably forms an addition compound with semicarbozide chloride and perhaps this is the reason that the earlier workers[4] claimed this compound as a ketonic acid.

Because of an appreciable amount of this acid in the oil; a complete study of its structure and properties is in progress and will be reported later.

The last fraction of the oil was composed of furocoumarins/coumarins. Only bergaptene and isopimpinellin were separated from this fraction by column chromatography and preparative TLC on the basis of UV light and identified by TLC and UV absorption against their standard samples. The rest of the coumarins have to be yet isolated and identified.

The aqueous distillate from the two oils, which appeared fluorescent under UV light, were extracted with diethyl ether. On removal of the solvent a brownish liquid was obtained. When allowed to stand it gave rise to needle-like crystals of bergaptene. The mother liquor was column chromatographed using silica gel and the components shown to mainly consist of seselin alongwith a small amount of thymol, bergaptene isopimpinellin, linalool, hydrocarbons and unidentified furocoumarins/coumarins.

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