Pakistan J. Sci. Ind. Res., Vol. 19, Nos. 5-6, October-December 1976

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

Part IV. *Apium graveolens* Linn. (Celery, Ajmodh) Seed Oil

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(Received August 4, 1975)

Abstract. The essential oil as well as the water cohobation oil of the seed of *Apium graveolens* grown in the Jhelum and Lahore districts has been studied for the first time with respect to its physicochemical characteristics and chemical composition. For the two areas the essential oil has been obtained in 2.5% and 2.6% yield while its composition is α-pinene (1.0, 0.5%), β-pinene (1.5, 0.8%), mycrene (3.1, 6.1%), limonene (35, 37%), *p*-cymene (3.1, 2.5%), β-elemene (3.5, 1.5%), β-caryophyllene (4.1, 3.1%), β-selinene (32.5, 28.5%), 3-isolbutylidene-3a,4-dihydrophthalide (0.7, 1.0%), carvone and dihydrocarvone (0.4, 1.0%), eudesmol (1.0, 0.5%), γ-terpineol (0.3, 0.5%), n-butylphthalide (5.0, 7.2%), and sedanonic anhydride (8.0, 7.9%) respectivley. The water cohobation, oils' yield and composition in the same order are 0.55% and 0.49% and α-pinene (traces, 0.5%), β-pinene (traces, 0.3%), myrcene (2.0, 3.5%, limonene (2.8, 3.0%), *p*-cymene(1.0, 1.2%), β-caryophyllene (2.4, 1.6%), humulene (1.4, 3.2%), β-selinene (1.3, 1.1%), 3-isobutylidene-3a.-4-dihydrophthalide (0.6, 0.3%), carvone and dihydrocarvone (0.7, 1.2%), n-butylphthalide (25.0, 20.0%), and sedanonic anhydride (60.0, 63.0%).

Apium graveolens (celery) is widely cultivated in the temperate zones of the world in general and in France, India, Holland, Hungary, China athe U.S.A. in particular. Distillation of the essential oil of the celery seed is commercially carried out in all these countries, but by far the major producer is almost certainly the United States, followed by France and the United Kingdom. In 1960, Arctander estimated that the annual world production of the oil from celery seed had increased considerably and stood at about 50 tons per annum.[1]

Celery is a known preventive of rheumatism and gout.[2] It is described in the local materia medica as deobstruent and resolvent. It is generally used internally as pactoral and as a tonic and carminative adjunct. Its seed are also administered as stimulant and cardiac tonic. They are used in bronchitis and asthma as antispasmodic and are also applied to some extent for liver and spleen disorders.

The essential oil of celery seed finds major application in the flavour industry, where it is used in the improvement of taste and aroma of prepared foods, soups, meats sauces, pickles and vegetable juices. The oil also finds minor utilization in both the perfumery and the pharmaceutical industries. In perfumery it has a rather powerful odour and if used judiciously it imparts a pleasant warm note. In pharmacy the oil supposedly exerts a sedative effect in certain preparations.

In spite of its commercial importance celery has so far received little attention as an industrial crop of Pakistan. The recent studies have, therefore, been carried out with a view to determining the quality and chemical composition of the essential oil of the celery of Pakistan, and indicating its qualitative and quantitative status in relation to similar oils produced in other countries. These are the first studies of this kind in so far as the local oil is concerned.

The species has so far been cultivated in Pakistan on a small scale, mainly in the Choa-Saiden Shah area in the Jhelum District. Its cultivation at these Laboratories, has, however, initially given a yield of 400 lb seed per acre in the present studies.

Experimental

Material. Fresh and mature seeds of celery cultivated at Choa-Saiden Shah in the Jhelum District and the PCSIR Laboratories experimental plots Lahore were used for the distillation of the essential oil.

Recovery of the Oils. The essential oil was recovered by dry steam distillation of the freshly ground 20–30 mesh seed according to the standard procedure.[3] The residual aqueous distillate was extracted twice with diethyl ether. The combined ether extracts were washed with water and dried (Na_2SO_4). The percentage yields of the essential oil and the water-cohobation oil and the distillation time are recorded in Tables 1 and 3 respectivley.

Methods

Physicochemical Characteristics. The instruments used in the determination of various characteristics of the oils and melting points of the solids in these studies have already been reported.[3] Chemical values of the oils were determined according to Guenther[4] and are also recorded in Tables 1 and 3.

Analysis of the Oil. The details of the method followed for the analysis have already been described.[3] Briefly, a weighed quantity of the oil (about 20 g) was loaded on a column (100×3.5 cm) packed with silica

gel (250 g). The hydrocarbon fraction was eluted with n-hexane and further resolved into terpenes and sesquiterpenes by gas–liquid chromatography using 3 m×3 mm copper column, packed with polyethylene glycol succinate (BDH) on Celite (60–80 mesh), nitrogen as the carrier gas and flame ionisation detector. The column temperature was maintained at 160°. The oxygenated components were eluted with 0.5, 0.8, 1.0, 10 and 50% diethyl ether in n-hexane and identified by IR comparison and conversion into known compounds.

The constituents of the hydrocarbon fraction as identified and determined by GLC and those of the oxygenated fractions as separated and estimated by column chromatography of both the oils are recorded in Tables 2 and 4.

Chemical Examination of the Water-Cohobation Oil. The water-cohobation oil was separated into neutral and acidic fractions by treatment with KOH.[5]

The oil (50 g) was refluxed for 3 hr with twice its weight of an aqueous solution of 25% KOH and then cooled. The unreacted material was removed with diethyl ether, washed with water and dried (Na_2SO_4) Removal of the solvent gave an oily neutral fraction (10 g, 20%) which consisted mainly of hydrocarbons alongwith carvone and dihydrocarvone in small amounts.

The alkali-soluble fractions was further separated into acids and lactones by treatment with sodium carbonate by the procedure as follows:

The alkali-soluble fraction was treateed with dilute sulphuric acid and the resulting oily layer was repeatedly extracted with diethyl ether. After removal of ether, a residue (37.6 g, 75.2%), smelling of celery was obtained. It was treated at 40° with three times of its weight of 10% aqueous sodium carbonate for 24 hr, cooled to room temperature and extracted with diethyl ether three time. The combined etheral extracts were washed with 5% sodim carbonate solution and then with water, and dried (Na_2SO_4) A lactone fraction (10.2 g, 20.4%) (95% n-butylphthalide) was obtained on removal of the solvent.

The sodium carbonate soluble matter on treatment with dilute sulphuric acid gave a light yellow butter-like semisolid mass (26.7 g, 53.4%) which on repeated crystallization from benzene and hexane gave an acid, m.p. 111–112°, oxime, m.p. 126–127°. Its IR (Fig.1) as well its m.p. and that of its oxime agreed well with those of sedanonic acid.

Preparation of Sedanolide. The method described

TABLE 1. YIELD AND PHYSICOCHEMICAL CONSTANTS OF THE CELERY ESSENTIAL OIL.

Constant	Essential oil from		Lit [7]
	Choa-Saiden Shah (Jhelum District)	Lab experimental plots	
Percentage yield	2·5 max	2·6 max	1·3–2·5
Time (hr)	10–12	10–12	12
Refractive index	$1·475^{34}$	$1·480^{30}$	$1·483^{20}_{20}$
Specific gravity	$0·837^{34}$	$0·842^{30}$	$0·882^{25}$
Optical rotation	+63° 14′	+60° 43′	+65° 46′
Acid value	1·3	0·8	2·47
Ester value	41·41	46·2	43·08

TABLE 3. YIELD AND PHYSICOCHEMICAL CONSTANTS OF THE CELERY WATER–COHOBATION OIL.

Constant	Water-cohobation oil from	
	Choa-Saiden Shah (Jhelum District)	Laboratories experimental plots
Yield (%)	0·55 msx	0·49
Time (hr)	10–12	10–12
Refractive index	$1·517^{16}$	$1·4992^{20}$
Specific gravity	$0·9444^{16}$	$0·9264^{20}$
Optical rotation	—25° 12′	—30° 52′
Acid value	5·6	4·2
Ester value	165	173

TABLE 2. COMPOSITION OF THE CELERY ESSENTIAL OIL.

Eluent	Constituents	Choa-Saiden Shah (Jhelum District)(%)	Lab. experimental plots (%)	Lit. [5,8,9]
n-Hexane	Hydrocarbons*	84·0	80·0	+
	α-Pinene	1·0	0·5	+
	β-Pinene	1·5	1·5	+
	Myrcene	3·1	6·1	+
	Limonene	35·0	37·0	+
	γ-Terpinene	—	—	+
	p-Cymene	3·1	2·5	+
	β-Elemene	3·5	1·5	+
	β-Caryophyllene	4·3	4·3	+
	β-Selinene	32·5	28·5	+
	Humulene	—	—	+
0·5% Diethyl ether in n-hexane	3-Isobutylidene-3a,4-dihydrophthalide	0·7	1·0	+
0·8% Diethyl ether in n-hexane	Carbonylic compounds	0·2	0·15	+
1·0% Diethyl ether in n-hexane	Carvone and dihydrocarvone	0·4	1·0	+
10% Diethyl ether in n-hexane	Edusmol	1·0	0·8	+
,,	γ-Terpineol	0·3	0·5	+
,,	Hydroxy compounds	0·5	0·8	+
50% Diethyl ether in n-hexane	n-Butylphthalide	5·0	7·2	—
,,	Sedanonic anhydride	3·0	7·9	—
Diethyl ether	Tarry material	1·0	1·0	—

* Resolved and estimated by GLC.

by Barton and Vries[6] was adopted for the conversion of sedanonic acid into sedanolide with b.p. 130–132° 0.8–1.0 mm, n_D^{20} 1.5000 (lit.[5] b.p. 185/17 mm n_D^{24} 5 F 1.49234).

Discussion

The yields and physicochemical properties of the essential oil obtained from the two places are comparable with those recorded for similar oils in literature (Table 1).[7] Qualitatively, the constituents of the Pakistani oil are largely the same as those of similar oils produced elsewhere and reported in literature (Table 2).

The hydrocarbons of the oil which amount to 84 and 80% for the two places were further resolved into terpenes and sesquiterpenes by GLC. The identity and percentage of the individual terpenes and sesquiterpenes as determined in those studies have also been recorded (Table 2). GLC of the terpenes fraction shows that α-pinene, β-pinene, myrcene and *p*-cymene are the most readily identifiable constituents of the essential oil. Limonene, the most abundant single constituent in the terpenic fraction, represents about 35–37% of the total oil. Three sesquiterpenes namely β-elemene, β-caryophyllene and β-selinene were identified in the sesquiterpenic fraction. Wilson[8] using GLC, reported the presence of α-pinene, β-pinene, myrcene, limonene, γ-terpinene, *p*-cymene, elemene, γ-caryophyllene and β-selinene in the American celery essential oil. Indian workers[11] have, however, reported the presence of only limonene and selinene in the Indian celery essential oil.

The oxygenated fraction consisted of 3-isobutylidene-3a, 4-dihydrophthalide, carvone, dihydrocarvone, leudesmol, γ-terpineol, n-butylphthalide and sedanonic anhydride.

TABLE 4. COMPOSITION OF THE CELERY WATER COHOBATION OIL.

Eluent	Constituents	Water-cohobation oil from Choa-Saidan Shah (Jhelum District) (%)	Water-cohobation oil from Lab experimental plots (%)
n-Hexane	Hydrocarbons*	12·0	15·0
	α-Pinene	traces	0·5
	β-Pinene	traces	0·3
	Myrcene	2·0	3·5
	Limonene	2·8	3·0
	p-Cymene	1·0	1·2
	β-Caryophyllene	2·4	1·6
	Humulene	1·4	3·2
	β-Selinene	1·3	1·1
	Unidentified sesquiterpene	1·3	0·6
0·5% Dietyl ether in n-hexen	3-Isoubylidene-3a,4-dihydrophthalide	6·0	0·3
1·0% Dietyl ether in hexane	Carvone and dihydrocarvone	0·7	1·2
50% Diethyl ether in n-hexane	n-Butylphthalide	25·0	20·0
,,	Sedanonic anhydride	60·0	63·0
Diethyl ether	Tarry material	2·0	2·0

* Resolved and estimated by GLC.

3-Isobutlylidene-3a,4-dihydrophthalide was identified by IR comparison. It could be a mixture of 3-isobutylidene-3a, 4-dihydrophthalide and 3-isovalidene 4-dihydrophthalide as both have identical IR and R_f values. Gold and Wilson[10] have reported the presence of four compounds which possessed a characteristic celery aroma and flavour. Out of these four, two compounds, viz., isobutylidenephthalide and isovalidenephthalide were not detected during the current studies possibly due to their being present in traces in these essential oils.

The hydroxy fraction of the oil consisted of alcohols which were further separated into eudesmol, α-terpineol and a mixture of two unidentified alcohols by column chromatography using activated alumina. Wilson[9] identified and estimated thirteen alcohols from his celery essential oil by GLC, but his material contained about 10–15% of alcohols in the total oil, whereas our material consisted of only 1.8 and 2.1% of alcohols in the total essential oils.

n-Butylphthalide was identified by conversion into a known solid derivative 2, 1′-hydroxy pentyl benzene alcohol, m.p. 70–72° (lit.[6] 73–74°). Sedanonic anhydride whose IR is shown here (Fig. 2). was characterised by conversion into known solid sedanonic acid from this essential oil by GLC has been reported in literature.[12] However, we could not separate this acid by column chromatography employing silica gel.

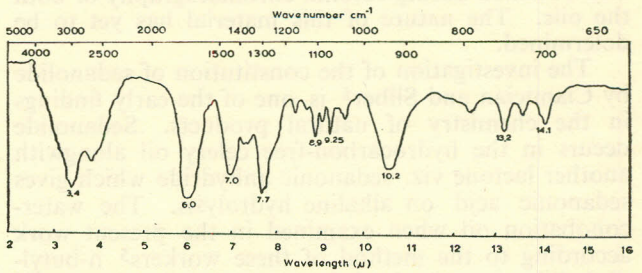

Fig. 1. Sedanonic acid.

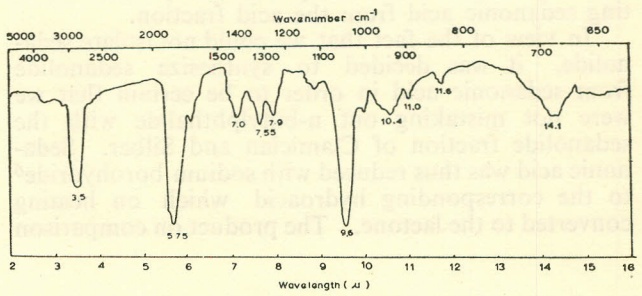

Fig. 2. Sedanonic anhydride.

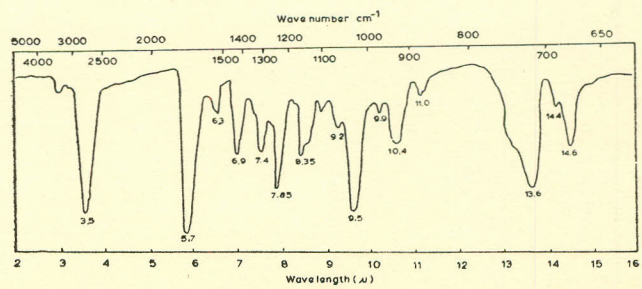

Fig. 3. Sedonolide.

Our work showing the presence of this acid as an anhydride is based on the chemical method already referred to in the Experimental.

The water–cohobation oil obtained as a result of ethereal extraction of the steam distillate has been investigated for the first time. The oil is brownish yellow with typical celery smell. The yield and physicochemical properties of the water–cohobation oil of both the places are markedly different from those of the essential oil, the former having higher specific gravity, higher acid and ester values and negative optical rotaion (Table 3). The IR spectra of the two oils are also different. While the essential oil contains mainly hydrocarbons, the water–cohobation oil is chiefly composed of oxygenated compounds. Nevertheless, none of the oils was completely free from either the hydrocarbons or the oxygenated compounds.

Like essential oil, the water–cohobation oil was also fractionated into individual constituents by column chromatography and GLC (Table 4). The water–cohobation oil has nearly the same constituents, in different proportions as that of the essential oil except that it contains humulene in addition to other sesquiterpenes in the hydrocarbon fraction and totally devoid of hydroxy compounds in the oxygenated fraction.

In the present studies, some tarry material was also obtained during column chromatography of both the oils. The nature of this material has yet to be determined.

The investigation of the constitution of sedanolide by Ciamician and Silber[5] is one of the early findings in the chemistry of natural products. Sedanolide occurs in the hydrocarbon-free celery oil alongwith another lactone viz. sedanonic anhydride which gives sedanonic acid on alkaline hydrolysis. The water-cohobation oil when examined in the present work according to the method of these workers[5] n-butylphthalide was obtained instead of sedanolide. No difficulty was, however, experienced by us in isolating sedanonic acid from the acid fraction.

In view of the fact that we could not isolate sedanolide, it was decided to synthesize sedanolide from sedanonic acid in order to be certain that we were not mistaking out n-butylphthalide with the sedanolide fraction of Ciamician and Silber. Sedanonic acid was thus reduced with sodium borohydride[6] to the corresponding hydroacid which on heating converted to the lactone. The product on comparison of its characteristics such as IR (Fig. 3) was found to be sedanolide in question.

Our inability in isolating sedanolide may, possibly be explained by assuming that environment might be affecting the chemical composition of the oil.

Before concluding we must state that reports on the analysis of the essential oil only have been made so far. We have, for the first time, analysed the water–cohobation oil and this investigation has clarified to some extent the composition of steam-volatile oils from celery seed. We conclude from the above studies that the essential oil of celery seed from plants growing in Pakistan possibly compares favourably with similar oils produced elsewhere in the world and that our oil is a potentially valuable commercial commodity.

Acknowledgements. We are grateful to the United States Department of Agriculture for financing this research under a PL-480 scheme and Mr. Abdul Waheed Sabir, our Botanist for the procurement of authentic material for the studies.

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