Registry No. Trypsin inhibitor, 9035-81-8; trypsin, 9002-07-7; Kunitz, 9088-41-9.

LITERATURE CITED

Ascenzi, P.; Menegatti, E.; Guarneri, M.; Bortolotti, F.; Antonini, E. *Biochemistry* **1982**, *21*, 2483–2490.
Baba, M.; Hamaguchi, K.; Ikenaka, T. *J. Biochem.* **1969**, *65*, 113–131.
Birk, Y. In "Bayer Symposium V: Proteinase Inhibitors"; Fritz, H., Tschesche, H., Greene, L. J., Truscheit, E., Eds.; Springer: Berlin, 1974; pp 355–361.
Blow, D. M.; Janin, J.; Sweet, R. M. *Nature (London)* **1974**, *249*, 54–57.
Burstein, E. A.; Vedenkina, N. S.; Ivkova, M. N. *Photochem. Photobiol.* **1973**, *18*, 263–279.
Cuatrecasas, P.; Anfinsen, C. B. *Methods Enzymol.* **1971**, *22*, 341–378.
Davis, B. J. *Ann. N. Y. Acad. Sci.* **1964**, *121*, 404–427.
Devenyl, T. *Acta Biochem. Biophys. Acad. Sci. Hung.* **1968**, *3*, 429–432.
Edelhoch, H. *Biochemistry* **1967**, *6*, 1948–1954.
Green, T. R.; Ryan, C. A. *Science (Washington, D.C.)* **1972**, *175*, 776–777.
Green, N. M.; Work, E. *Biochem. J.* **1953**, *54*, 347–352.
Ikeda, K.; Hamaguchi, K.; Yamamoto, M.; Ikenaka, T. *J. Biochem.* **1968**, *63*, 521–531.
Jirgensons, B. In "Optical Activity of Proteins and other Macromolecules": Springer-Verlag: Berlin, 1973.
Jirgensons, B. *Biochem. Biophys. Acta* **1978**, *534*, 123–131.
Jirgensons, B.; Kawabata, M.; Capetillo, S. *Makromol. Chem.* **1969**, *125*, 126–135.
Kassel, B. *Methods Enzymol.* **1970**, *19*, 844–852.
Koide, T.; Ikenaka, T. *Eur. J. Biochem.* **1977**, *32*, 417–431.
Kronman, M. M.; Holmes, L. G. *Photochem. Photobiol.* **1971**, *14*, 113–134.
Laemmly, U. K. *Nature (London)* **1970**, *227*, 680–685.
Lan, A.; Ako, H.; Werner-Washburne, M. *Biochem. Biophys. Res. Commun.* **1980**, *92*, 1243–1249.
Laskowski, M., Jr.; Kato, I. *Ann. Rev. Biochem.* **1980**, *49*, 593–626.
Lutz, H. P.; Luisi, P. L. *Helv. Chim. Acta* **1983**, *66*, 1929–1935.
Manjunath, N. H.; Veerabhadrappa, P. S.; Virupaksha, T. K. *Phytochemistry* **1983**, *22*, 2349–2357.
Quast, V.; Engel, J.; Steffen, E.; Tschesche, H.; Kupfer, S. *Biochemistry* **1978**, *17*, 1675–1682.
Richardson, M. *Phytochemistry* **1977**, *16*, 159–169.
Richardson, M. *Food Chem.* **1981**, *6*, 235–253.
Rizzo, V.; Luisi, P. L. *Biopolymers* **1977**, *16*, 437–448.
Ryan, C. A. In "The Biochemistry of Plants"; Academic Press: New York, 1981; Vol. 9, pp 351–370.
Steiner, R. F.; Edelhoch, H. *Nature (London)* **1961**, *192*, 873–874.
Sweet, R. M.; Wright, H. T.; Janin, J.; Chothia, C. H.; Blow, D. M. *Biochemistry* **1974**, *13*, 4212–4228.
Tamura, Y.; Jirgensons, B. *Arch. Biochem. Biophys.* **1980**, *199*, 413–419.
Toniolo, C.; Bonora, G. M.; Vita, C.; Fontana, A. *Biochem. Biophys. Acta* **1978**, *532*, 327–336.
Walde, P.; Luisi, P. L.; Palmieri, S. *J. Agric. Food Chem.* **1984**, *32*, 322–329.
Woody, R. W. *Biopolymers* **1978**, *17*, 1451–1467.

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Composition of the Essential Oil from *Asarum canadense*

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The steam distilled oil obtained from the ground root material of *Asarum canadense* (family Aristolochiaceae) is known in the flavor and fragrance industry as Canadian snakeroot oil (wild ginger). The oil has a spicy odor and flavor and is used in many flavor preparations. We have investigated this essential oil in order to determine its composition. A combination of chromatographic and spectroscopic methods has been used to characterize the oil. We have been able to characterize greater than 90% of the oil and have identified fifty one individual components, of which thirty seven have not previously been reported.

Oil of Canadian snakeroot (wild ginger) is produced by steam distillation of the dried root material of *Asarum canadense* (family Aristolochiaceae). The oil which is chiefly used in flavor preparations, has a strong spicy odor and flavor, the physical properties of which have been described by Guenther (1952). We have undertaken an investigation of this oil in order to determine its composition. Previous investigators, Power and Lees (1902), Ikede et al. (1962), and Bauer et al. (1967) have reported some of the components in this oil. Here now we report a more comprehensive list of the constituents of this essential oil.

EXPERIMENTAL SECTION

The essential oil was obtained by the steam distillation of the finely ground root material of *Asarum canadense*. The total yield of oil obtained in this manner was 3.0%. The individual components of the oil were separated and identified by using the following general scheme.

The crude oil was first distilled under vacuum (0.5 mm) through a column packed with glass coils. The pot temperature was kept at 110 °C. This divided the oil into two general fractions, the distilled material and the pot residue, each constituting about 50% of the oil. Each of these fractions was then further separated in the following manner. The distillate was first fractionated by spinning band distillation under vacuum and then further separated by preparative gas chromatography. The pot residue likewise was further separated by column liquid chromatography, followed by HPLC, and if needed, preparative gas chromatography.

Identification of each of the components in each fraction was first done by GC–MS combined with computer matching of the mass spectrum with that of materials present in our library. Further proof of each component's identification was accomplished by the comparison of the retention index data obtained on two capillary GC columns

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Table I. Components Identified in Canadian Snakeroot Oil

no.[a]	component	rel %[b]	DB-1[c]	CBWX[c]	ID methods[d]
	3-methylbutanal	<0.05			MS
	3-methylbutanol	<0.05			MS
	pentanol	<0.05			MS
	3-methyl-2-butenal	<0.05			MS
	2-methyltetrahydro-3-furanone	<0.05			MS
	hexanal	<0.05			MS
	furfural	<0.05			MS
	4-hydroxy-4-methyl-2-pentanone	<0.05			MS
	3-methylcyclopentanone	<0.05			MS
	acetylfuran	<0.05			MS
	2,6-dimethylpyrazine	<0.05			MS
	5-methylfurfural	<0.05			MS
	benzaldehyde	<0.05			MS
1	α-pinene[e]	1.50	5.63	4.0	MS
2	camphene[e]	0.62	5.80	4.37	MS
3	sabinene[e]	0.77	5.99		MS
4	β-pinene[e]	1.35	6.08	4.75	MS
5	myrcene[e]	0.98	6.08		MS, r_t
	1,4-cineole	<0.05			MS
6	*p*-cymene	0.09	6.44	6.40	MS
7	limonene[e,f]	0.64	6.55	5.68	MS
8	1,8-cineole	0.43	6.55	5.77	MS
	terpinolene	<0.05			MS
	linalool oxide (*cis*-furanoid)	<0.05			MS
	linalool oxide (*trans*-furanoid)	<0.05			MS
9	linalool[f]	4.99	7.12	9.11	MS
	hotrienol	<0.05	7.14	9.72	MS, r_t, synthesis, ^{1}H NMR, ^{13}C NMR
	trans-*p*-menthen-1-ol	<0.05			MS
	pinocarveol	<0.05	7.60	9.97	MS, r_t
10	borneol	0.07	7.76	10.57	MS, r_t
11	terpinen-4-ol	0.43	7.93	9.56	MS
12	α-terpineol[f]	1.54	8.02	10.48	MS
	γ-terpineol	<0.05			MS
	verbenone	<0.05			MS
	methylchavicol	<0.05	8.04	10.2	MS, r_t
	myrtenal	<0.05			MS
13	thymyl methyl ether	0.30	8.39		MS
14	geraniol[f]	0.84	8.64	11.88	MS
15	linalyl acetate[f]	28.02	8.72	9.29	MS
16	bornyl acetate[f]	2.02	9.07	9.37	MS
	cuminic alcohol	<0.05			MS
17	eugenol	0.60	9.60	14.89	MS
18	neryl acetate	0.69	9.69	10.87	MS
19	geranyl acetate	1.36	9.84	11.23	MS
20	methyleugenol[f]	36.05	9.90	13.55	MS
	methylisoeugenol	<0.05			MS
21	elemicin[f]	1.76	11.47	15.56	MS, r_t
22	unknown	0.83	11.89		MS - M^+ 208
23	junenol	1.23	12.48	13.8	MS, ^{1}H NMR, ^{13}C NMR[g]
24	unknown	0.30	12.50		MS - M^+ 222
25	*n*-butylphthalide	0.23	12.45	18.06	MS, r_t
26	*trans*-isoelemicin	0.92	12.40	17.60	MS, r_t, ^{1}H NMR, ^{13}C NMR, synthesis
27	unknown (aromatic)	0.83	12.75		MS - M^+ 238
28	unknown (sesquiterpenoid)	0.85	12.85		MS - M^+ 238
29	sedanolide	0.28	13.19		MS, ^{1}H NMR, ^{13}C NMR
30	artistolone[f]	0.79	13.71	17.1	MS, ^{1}H NMR, ^{13}C NMR
31	unknown	1.09	13.72	17.1	MS - M^+ 218

[a] No. refers to peaks numbered in Figure 1. [b] Rel % was calculated by using the GC FID intergration areas. [c] Retention indices were calculated based on the standard ethyl esters, C-4 through C-18. [d] Identification methods: MS, library match of component from GC–MS; ^{1}H NMR spectra run at 250 MHz; ^{13}C NMR spectra run at 62.9 MHz; synthesis, material was synthesized; r_t, identity confirmed by co-injection of authentic material. [e] Component reported by Ikede et al. (1962). [f] Component reported by Bauer et al. (1967). [g] Identified by comparison to literature spectra: Shaligraam et al. (1962); Thomas et al. (1976).

(DB-1, Carbowax) or by co-injection with the authentic material. When no GC–MS library match could be obtained, the component was then isolated by either preparative HPLC or GC. The structure of the isolated component was then determined by interpretation of its ^{1}H NMR, ^{13}C NMR, and mass spectrum. The structure was then confirmed by comparison to literature spectra. Where possible the material was synthesized in order to confirm its identity.

INSTRUMENTATION

Column chromatography was performed on silica gel-60 (EM reagents, 230–400 mesh) under low pressure (20 psi) with hexane–ethyl acetate or hexane–ethyl ether as solvent.

High performance liquid chromatography (HPLC) was performed by using a DuPont System 8800 liquid chromatograph, equipped with a four solvent gradient, a UV spectrophotometer, and a refractive index detector. Chromatography was accomplished by using either an Altex SiO_2 semiprep column (10 × 250 mm 5μ μ-porasil) or a Whatman C-18 semiprep column (9.4 × 250 mm 10μ partisil ODS-II). The solvents used were all HPLC grade (Burdick & Jackson) and degassed with helium before use. Collections were carried out automatically with a Siemens

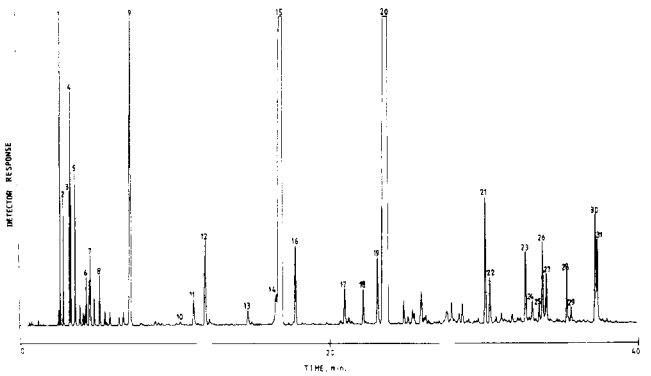

Figure 1. Capillary gas chromatograph trace of oil of Canadian snakeroot. Peak numbers refer to components listed in Table I (GC column SE-30, 30m × 0.32 mm, temperature programmed 80–220 °C at 4 °C/min).

fraction collector set in the time collection mode.

Preparative gas chromatography was carried out either on an OV-101 or Carbowax $^1/_4$ in. glass column with a Perkin-Elmer (Model 900) gas chromatograph equipped with a flame ionization detector (FID); the effluent was split between the FID and collection port (split ratio 1:9).

Analytical gas chromatography retention time measurements were performed on either a Perkin-Elmer Sigma 2000 gas chromatograph or a Perkin-Elmer Sigma-2 gas chromatograph with both DB-1 (J&W Scientific, 30m × 0.32 mm, 1μ film) and Carbowax-20M capillary columns (J&W Scientific, 30m × 0.32 mm). Some analytical chromatograms were run on a Perkin-Elmer Sigma 2000 gas chromatograph with an SE-30 capillary column (J&W Scientific, 30m × 0.32 mm).

Mass spectra were taken with a Hewlett-Packard Model 5985 mass spectrometer equipped with an H-P gas chromatograph with a DB-1 GC column (J&W Scientific 30m × 0.25 mm, 1μ film).

Proton NMR spectra and carbon-13 NMR spectra were obtained with a Bruker WM-250 NMR spectrometer (250 MHz for proton and 62.9 MHz for carbon). All spectra were obtained in $CDCl_3$ and were referenced to the solvent peak (^{1}H NMR 7.25 ppm, ^{13}C NMR 77.0 ppm).

RESULTS

Table I indicates the components we have identified in oil of Canadian snakeroot. The components have been listed in order of their elution from a DB-1 gas chromatograph column and are represented by the trace in Figure 1. For each component, the methods of identification have been given as well as its percent composition in the oil. In all identified components the retention index or relative retention time was used as one method of identification.

Registry No. 3-Methylbutanal, 590-86-3; 3-methylbutanol, 123-51-3; pentanol, 71-41-0; 3-methyl-2-butenal, 107-86-8; 5-methyltetrahydro-3-furanone, 3188-00-9; hexanal, 66-25-1; furfural, 98-01-1; 8-hydroxy-4-methyl-2-pentanone, 123-42-2; 3-methylcyclopentanone, 1757-42-2; acetylfuran, 1192-62-7; 2,6-dimethylpyrazine, 108-50-9; 5-methylfurfural, 620-02-0; benzaldehyde, 100-52-7; α-pinene, 80-56-8; camphene, 79-92-5; sabinene, 3387-41-5; β-pinene, 127-91-3; myrcene, 123-35-3; 1,4-cineole, 470-67-7; *p*-cymene, 99-87-6; limonene, 138-86-3; 1,8-cineole, 470-82-6; terpinolene, 586-62-9; *cis*-linalool oxide, 5989-33-3; *trans*-linalool oxide, 34995-77-2; linalool, 78-70-6; hotrienol, 20053-88-7; pinocarveol, 5947-36-4; borneol, 507-70-0; terpinen-4-ol, 562-74-3; α-terpineol, 98-55-5; γ-terpineol, 586-81-2; verbenone, 80-57-9; methylchavicol, 140-67-0; myrtenal, 564-94-3; thymyl methyl ether, 1076-56-8; geraniol, 106-24-1; linalyl acetate, 115-95-7; bornyl acetate, 76-49-3; cuminic alcohol, 536-60-7; eugenol, 97-53-0; neryl acetate, 141-12-8; geranyl acetate, 105-87-3; methyleugenol, 93-15-2; methylisoeugenol, 93-16-3; elemicin, 487-11-6; junenol, 472-07-1; *n*-butylphthalide, 6066-49-5; *trans*-isoelemicin, 5273-85-8; sedanolide, 6415-59-4; aristolone, 6831-17-0.

LITERATURE CITED

Bauer, L.; Bell, C. L.; Gearien, J. E.; Takeda, H. *J. Pharm. Sci.* **1967**, *56*, 336–343.

Guenther, E. "The Essential Oils"; D. Van Nostrand Company, Inc.: New York, 1952; Vol. VI, p 145–147.

Ikeda, R. M.; Stanley, W. L.; Vannier, S. H.; Spitler, E. M. *J. Food Sci.* **1962**, *27*, 455–458.

Power, F. B.; Lees, F. H. *J. Chem. Soc.* **1902**, *81*, 58.

Shalligram, A. M.; Rao, A. S.; Bhattacharyya, C. S. *Tetrahedron* **1962**, *18*, 969–977.

Thomas, A. F.; Ozainne, M.; Decorzant, R.; Naf, F.; Lukacs, G. *Tetrahedron* **1976**, *32*, 2261–2264.

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