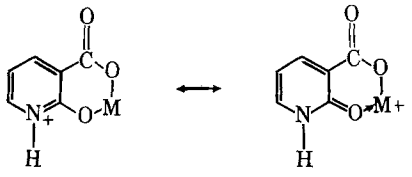
and the π-deficient character of the pyridine ring.



I II

Biologic Activities—Results of analgesic testing[1] of the 3,6-dialkyl-substituted salicylic acids showed that both the 3,6-dimethyl and 3-methyl-6-isopropyl derivatives had about 40% greater activity than aspirin, while the 3-*tert*-butyl-6-methyl derivative had about 20% more activity. Analgesic testing was carried out in rats (20 per compound) at compound dosages of 300 mg./Kg. by heating the rat's tail. Because of the lower metal-binding abilities of these acids in water in comparison to that of salicylic acid, it cannot be concluded that greater avidity for metal ions confers greater analgesic effects. It is significant, however, that *o*-thymotic acid also claimed to have a greater analgesic effect than salicylate (19) has also somewhat lower metal-binding constants (1).

Tests for anti-inflammatory and antierythemic effects[2] were also positive for 3,6-dimethylsalicylic acid. In this test, carried out by the method of Winder *et al.* (20), a dosage level of 80 mg./Kg. in guinea pigs produced a decrease in response to U.V.-induced erythema in $^7/_8$ animals, compared to the same response from 100 mg./Kg. of aspirin. In comparison, 3,5-diisopropylsalicylic acid (1) reduced these effects in only $^2/_8$ animals, and both 2-hydroxypyridine-3-carboxylic acid and 3-hydroxypyridine-4-carboxylic acid showed no effects at 80 mg./Kg.

[1] Carried out under the direction of Dr. Howard J. Jenkins.
[2] These results were supplied through the courtesy of Dr. Blaine M. Sutton, Smith Kline & French Laboratories.

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Constituents of the Rhizome of *Asarum canadense*

By LUDWIG BAUER, CHARLES L. BELL, JAMES E. GEARIEN, and HIROSHI TAKEDA

A pentane extract from the rhizomes of *Asarum canadense* was separated first by means of steam distillation, and then by column and gas chromatography. From the steam-volatile oil the following constituents were isolated (percentages are shown in parentheses): methyleugenol (44.5), linalyl acetate (41.1), geraniol (7.4), linalool (5.3), limonene (0.8), α-terpineol (0.4), bornyl acetate (0.3), aristolone (0.1), elemicin (0.1), 2,3,4,5-tetramethoxyallylbenzene (0.05), 2,4-dimethoxycinnamaldehyde (0.05), and two unidentified compounds (0.01 each). The steam-nonvolatile residue contained some of the above and also β-sitosterol.

THE FRAGRANT essential oil of *Asarum canadense* (Canada snakeroot, wild ginger) has not been investigated since 1902 when Power reported it to contain mainly methyleugenol (3,4-dimethoxyallylbenzene), pinene, and (after saponification) linalool, borneol, terpineol, geraniol, and several unidentified compounds (1, 2). This work set out to re-examine this oil and, in particular, search for minor constituents. Instead of subjecting the ground rhizomes immediately to steam distillation (1, 2), it was advantageous to obtain a pentane extract first which then was separated into a steam-volatile fraction (oil *A*) and a residue (oil *B*). Examination of oil *A*, by gas chromatography (GC) revealed the presence of at least 13 components (Fig. 1, Table

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The authors acknowledge with thanks receipt of the following authentic samples: aristolone from Dr. N. Soma, Takamine Laboratory, Osaka, Japan; ferulone from Dr. A. Marsili, University of Pisa, Pisa, Italy; elimicin from Dr. A. T. Shulgin, Dow Chemical Co., Walnut Creek, Calif.; 2,3,4,5-tetramethoxyallylbenzene from Dr. E. Stahl, Universität des Saarlandes, Saarbrücken, Germany; β-sitosterol from Dr. Percy L. Julian, Julian Associates, Inc., Franklin Park, Ill.

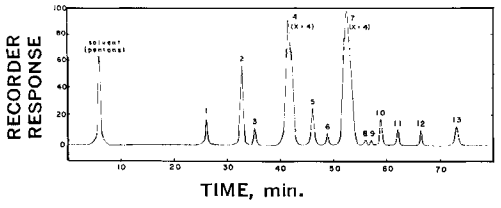


Fig. 1—Vapor-phase chromatogram of the steam-volatile oil, A, from a pentane extract of the rhizomes of Asarum canadense *(20 ft. × 3/8-in. o.d. coiled aluminum column), packed with 20% SE-30 on Chromosorb W, nonlinearly programmed from 90 to 250° over 54 min. using the Autoprep model A-700, the helium flow rate being 200 ml. per minute measured at the outlet of the apparatus. Table I lists the constituents associated with the numbered peaks.*

I) of which two were present in about equal amounts and composed 85% of the oil. Although column chromatography failed to separate the components of oil *A*, this technique served to concentrate the minor constituents somewhat more effectively (see Table II). Each eluate was resolved by means of preparative GC, and the pure components were identified by comparing their various spectra with those of authentic samples and those in the literature. The constituents of the steam-volatile oil *A* are discussed first (Fig. 1, Table I).

DISCUSSION

Monoterpene Hydrocarbons—The first fraction in the GC of *A* was identified as limonene by its mass spectrum (molecular ion *m/e* 136, base peak *m/e* 68), identical to the published spectrum (3), and to that of an authentic sample of dipentene. Since pinene was reported to be present in this oil (2), attempts were made to find this hydrocarbon by examining the mass spectra of all GC fractions before and after chromatography of *A*. Although the retention times (under the conditions for the separation of *A*) and mass spectra of pure α- and β-pinenes were in hand, these hydrocarbons could not be detected. It was observed that after chromatography of *A*, β-myrcene was found in fraction *A*-I which was identified by its NMR (4, 5) and mass spectra (3, 5, 6). This hydrocarbon was definitely not present in oil *A* and it was found that at the same time, the amount of linalyl acetate in *A* decreased from 44 in *A* to 1.8% after chromatography. The suspicion that linalyl acetate underwent elimination was confirmed when a sample of the ester was chromatographed under similar conditions to give β-myrcene, linalool, and unchanged ester in the ratio of 2:1:2. It was interesting to note that alumina acted as a basic catalyst promoting the elimination of acetic acid from linalyl acetate to give β-myrcene only, uncontaminated by *cis*- and *trans*-β-ocimene (5). This reaction is so different from the catalytic pyrolysis of linalyl acetate (7) over acid-washed Chromosorb P at 140–150° to give β-myrcene, *cis*- and *trans*-β-ocimene, and dipentene in the ratio of 43:20:35:2. The conversion of the linalyl acetate to linalool during the chromatography can be explained by either a hydrolytic process due to adsorbed water or by ester interchange during elution with a mixture of chloroform and ethanol.

TABLE I—COMPONENTS OF THE VAPOR-PHASE CHROMATOGRAM SHOWN IN FIG. 1

Peak No.	Component	Retention Time, min.	Compn. Vol., %
1	Limonene	25.4	0.8
2	Linalool	32.5	5.3
3	α-Terpineol	35.0	0.4
4	Linalyl acetate	41.6	41.1
5	Geraniol	46.0	7.2
6	Bornyl acetate	48.5	0.3
7	Methyleugenol	52.0	44.5
8	Unidentified	56.0	0.01
9	Unidentified	57.0	0.01
10	Elemicin	58.5	0.1
11	3,4-Dimethoxycinnamaldehyde	62.0	0.05
12	2,3,4,5-Tetramethoxyallylbenzene	66.6	0.05
13	Aristolone	73.0	0.1

TABLE II—COLUMN CHROMATOGRAPHY OF OIL *A*

Fraction	Eluting Solvent	Yield, Gm.	Components	Percentage, from GC
A-I	Pentane	23	β-Myrcene	30
			Limonene	25
			Geraniol	10
			Bornyl acetate	5
			Methyleugenol	25
A-II	Pentane–benzene (9:1)	7	Geraniol	1
			Bornyl acetate	1
			Methyleugenol	95
			3,4-Dimethoxycinnamaldehyde	3
A-III	Benzene	5	Linalool	10
			Geraniol	2.5
			Bornyl acetate	2.5
			Methyleugenol	80
			3,4-Dimethoxycinnamaldehyde	5
A-IV	Benzene–ether (9:1)	3.9	Linalool	30
			Methyleugenol	60
			Elemicin	6
			2,3,4,5-Tetramethoxyallylbenzene	4
A-V	Ether	2.0	Linalool	45
			α-Terpineol	35
			Methyleugenol	5
			Aristolone	5
A-VI	Ether–chloroform (9:1)	1.0	Linalool	40
			α-Terpineol	35
			Aristolone	5
A-VII	Chloroform	1.0	Linalool	80
			α-Terpineol	15
A-VIII	Chloroform–ethanol (9:1)	8.0	Linalool	40
			α-Terpineol	10
			Linalyl acetate	10
			Geraniol	40

Monoterpene Alcohols and Esters—In identifying these components, it was possible to make use of the recently published mass spectra of a number of monoterpene alcohols and esters (8, 9). Cognizance was taken of the fact that in a number of instances the mass spectra of alcohols or their esters did not show the parent peak (8), the first peak for the alcohols being at M-18 and for the esters being either (M-RCO-1) or at (M-RCO_2H) (9).

Of the terpene alcohols in the steam-volatile oil *A*, geraniol was found in largest abundance and

was identified by its mass spectrum, which was identical to that of a purified authentic specimen and to the published spectrum (6, 8). Its NMR spectrum ($CDCl_3$) also matched the published one (10), except it was possible to observe the exchangeable OH proton as a singlet at 2.53 δ. α-Terpineol was also present and was identified by its mass spectrum (8) and its NMR spectrum ($CDCl_3$) which showed the vinyl proton at 5.48 δ, a singlet for one CH_3 at 1.75 δ, one for two CH_3's at 1.27 δ. In the steam-volatile oil *A*, linalool was found only in about 4%, but after chromatography its percentage rose to 15.6% (see above). The NMR spectrum of linalool in $CDCl_3$ showed three CH_3 singlets at 1.26, 1.60, and 1.68 δ, the exchangeable OH proton at 2.05 δ, and the alkene protons as complex multiplets between 4.9 and 6.3 δ. The mass spectrum of this sample of linalool also showed a peak at *m/e* 155 (M + 1) which had been noted previously (8) and been the subject of considerable discussion.[1]

Linalyl acetate was the major ester fraction of this essential oil and was found to be quite stable to steam distillation and to GC under the conditions described, but did react on alumina (see above). Its mass spectrum was not included among the terpene esters studied by Sydow (9) and is appended (Fig. 2). Like most terpene esters, it lacked the molecular ion peak at *m/e* 196, and the first fragment appeared at *m/e* 136 (M-60, M-CH_3-CO_2H). At first glance the fragmentation pattern appeared almost like that of β-myrcene—with some additional peaks and changes in relative intensities (see Table III). The peak *m/e* 60 is attributed to acetic acid, a fragment which was observed sometimes in the mass spectra of terpene acetates (9).

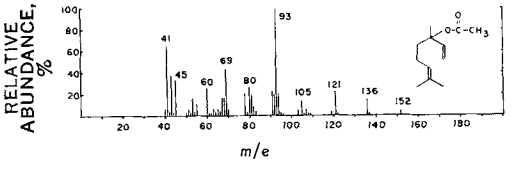


Fig. 2—The mass spectrum of linalyl acetate.

The only other ester which was isolated was bornyl acetate whose NMR and mass spectra matched those of an authentic sample. Although the mass spectra of bornyl and isobornyl acetates are almost identical (9), Biemann (11) was able to distinguish between these two isomers on the basis of the ratio of the abundance of the M and (M-60) ions. The identity of the ester isolated here was established here on the basis of its NMR spectrum ($CDCl_3$), which showed the methine proton as a doublet of quartets at 4.91 δ [previously reported at 5.19 δ, in CCl_4 (12)] which distinguished this ester from isobornyl acetate. Furthermore, the infrared spectrum of the isolated ester matched that of bornyl rather than isobornyl acetate, particularly due to differences in the 1000–1100 cm.$^{-1}$ region.

Table III—Mass Spectra of Linalyl Acetate, Linalool, and β-Myrcene

Linalyl Acetate (Mol. Wt. 196)		Linalool (Mol. Wt. 154)		β-Myrcene (Mol. Wt. 136)	
m/e	% Σ_{40}	*m/e*	% Σ_{40}	*m/e*	% Σ_{40}
40	0.6	41	8.1	40	1.3
41	10.4	43	6.3	41	14.6
42	0.3	53	1.7	42	1.4
43	6.1	54	1.1	43	2.6
44	0.3	55	6.3	44	2.6
45	5.2	56	1.5	51	1.7
50	0.3	57	0.3	53	2.3
51	0.6	58	0.3	55	1.7
52	0.3	59	2.5	64	0.8
53	2.4	67	1.7	65	1.4
54	0.3	68	2.0	67	2.9
55	1.6	69	7.8	68	2.0
60	4.0	71	10.8	69	12.0
61	0.3	72	0.5	70	1.7
62	0.3	77	2.0	77	4.1
63	0.8	79	2.5	78	0.8
64	0.4	80	4.5	79	5.8
65	0.8	81	2.0	80	3.5
66	0.4	82	0.3	81	0.8
67	2.4	83	1.7	82	0.8
68	2.4	91	1.0	91	4.4
69	6.9	92	2.5	92	3.5
70	0.6	93	12.6	93	13.1
78	3.2	94	2.5	94	3.5
79	0.3	95	0.7	96	1.1
80	4.0	96	1.6	105	1.0
81	3.2	97	0.3	107	1.0
82	1.3	105	2.2	121	3.2
83	0.4	106	0.3	136	2.9
91	3.5	107	1.7	137	0.5
92	3.2	108	0.5	...	...
93	16.0	109	1.2	...	...
94	3.2	111	0.7	...	...
95	0.4	120	0.3	...	...
96	0.3	121	3.0	...	...
103	0.6	135	0.3	...	...
105	2.1	136	2.5	...	...
106	0.3	139	0.3	...	...
107	0.8	155	0.5	...	...
108	0.3	...	...	...	...
109	0.3	...	...	...	...
119	0.4	...	...	...	...
121	3.5	...	...	...	...
122	0.3	...	...	...	...
136	2.4	...	...	...	...
137	0.3	...	...	...	...
152	0.6	...	...	...	...

Aromatic Compounds—Since a number of different alkoxy substituted allyl- and propenylbenzenes have been discovered in several *Asarum* species (13), *e.g.*, asarone [*trans*-1-(2,4,5-trimethoxyphenyl)-1-propene] and *trans*-methylisoeugenol, the predominant ethers in *Asarum europeum* (14), safrole, the principal component of *A. arifolium*, and mainly methyleugenol in *A. caduatum* and *canadense*, a particular effort was made to determine the nature of the aromatic constituents in *A. canadense*.

The major component of the steam-volatile oil *A* was proved to be methyleugenol whose spectra matched those of an authentic sample. It was oxidized by potassium permanganate to veratric acid (see under *Experimental* for the oxidation of elemicin). Its NMR spectrum (neat) showed the benzyl protons as a doublet (with fine structure) at 3.27 δ (J = 6 c.p.s.), the two OCH_3 resonances at

[1] It was suggested by Willhalm, B., Thomas, A. F., and Stoll, M., *Acta Chem. Scand.*, **18**, 1573(1964), that the peak *m/e* 155 stems from contamination of samples of linalool by (*cis* and *trans*) linalool oxides which are almost impossible to separate from the alcohol by chromatography. Since the mass spectra of the oxides [Felix, D., Melera, A., Seibl, J., and Kovats, E., *Helv. Chim. Acta*, **46**, 1513(1963)] contain fragments also present in the mass spectrum of linalool, the origin of the *m/e* 155 peak remains to be discovered.

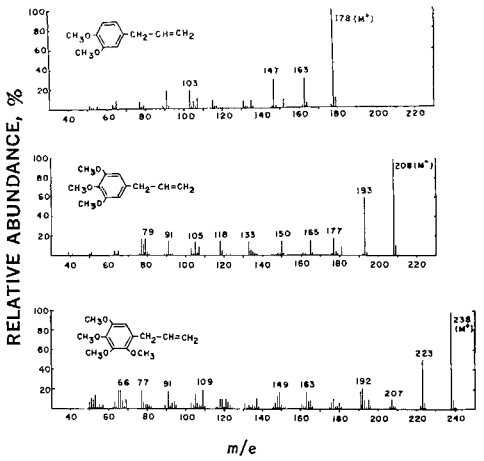


Fig. 3—The mass spectra of methyleugenol (top), elemicin (middle), and 2,3,4,5-tetramethoxyallylbenzene (bottom).

m/e 135.[2] The loss of a methoxy radical from the molecular ion showed the peak at *m/e* 147 (confirmed by the metastable ion at 69.5). The formation of a fragment *m/e* 151 by the loss of a vinyl radical from the molecular ion (M-27) would be expected to produce a substituted tropylium ion whose stability would overcome the relatively unstable vinyl radical. It should be noted that the mass spectrum of methylisoeugenol was quite similar to that of methyleugenol except for the absence of the peak at *m/e* 151 (M-27) in the former.

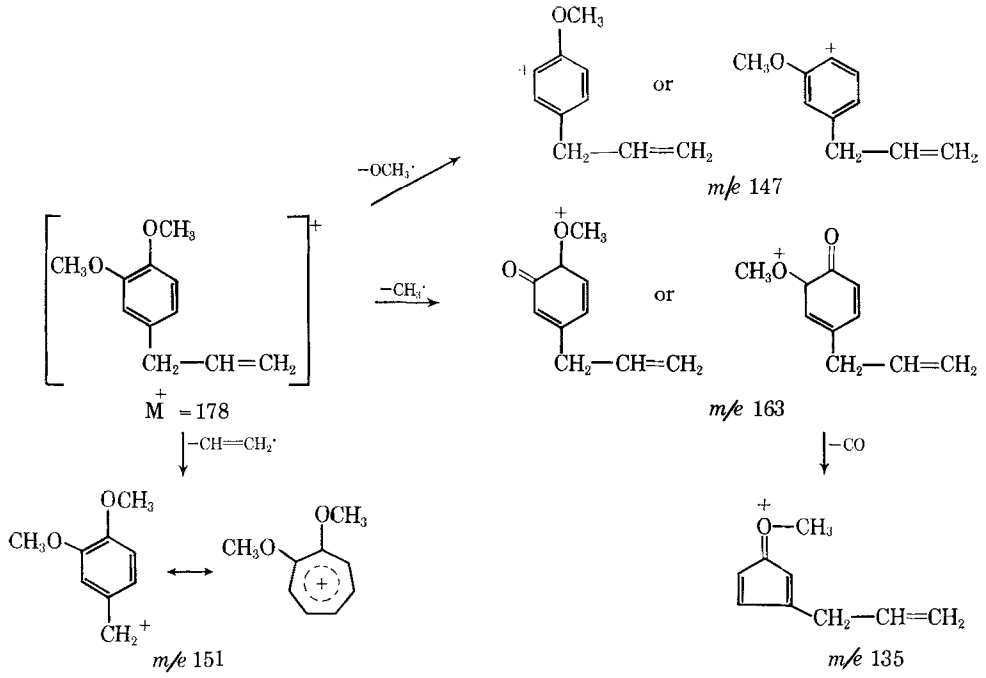
A small amount of elemicin (3,4,5-trimethoxyallylbenzene) was isolated from oil *A* and was identical to an authentic sample (15). Its structure was proved also by permanganate oxidation to 3,4,5-trimethoxybenzoic acid. Its NMR spectrum ($CDCl_3$) showed one aromatic proton at 6.41δ and the nine OCH_3 protons as a singlet at 3.82δ. The mass spectrum of elemicin (Fig. 3, Table IV) showed that the parent peak (208) was the base peak and the next most intense peak (61%) was *m/e* 193 (M-15) due to loss of a methyl radical,

TABLE IV—MASS SPECTRA OF METHYLEUGENOL, ELEMICIN, 2,3,4,5-TETRAMETHOXYALLYLBENZENE, AND 3,4-DIMETHOXYCINNAMALDEHYDE

Methyleugenol		Elemicin		2,3,4,5-Tetramethoxyallylbenzene		3,4-Dimethoxycinnamaldehyde	
m/e	% Σ_{40}	*m/e*	% Σ_{40}	*m/e*	% Σ_{40}	*m/e*	% Σ_{40}
51	1.8	51	1.3	51	1.5	51	0.9
65	1.8	63	1.3	65	2.7	52	2.5
77	2.4	65	1.3	66	3.0	53	1.1
79	1.8	77	3.5	67	1.4	63	0.9
91	4.8	78	1.7	77	2.7	64	1.7
103	4.8	79	3.9	78	1.1	66	1.1
105	1.8	91	3.5	91	2.1	77	4.3
107	3.3	103	1.5	94	1.1	78	2.7
115	2.1	105	2.8	103	1.1	89	1.7
131	1.8	107	1.5	105	1.9	91	3.6
135	2.4	118	3.5	109	2.2	102	1.1
147	8.4	119	1.3	118	1.1	103	2.5
151	4.2	133	3.2	119	1.1	106	1.4
163	8.4	134	1.3	121	1.6	118	1.7
178M+	30.0	135	1.3	137	1.8	121	3.6
179	3.6	150	3.0	148	1.8	123	1.1
180	0.6	151	1.1	149	2.1	132	0.9
...	...	161	1.1	163	3.0	133	1.7
...	...	165	3.0	164	1.4	138	2.1
...	...	177	3.5	165	1.4	149	3.6
...	...	178	1.1	177	1.8	151	1.1
...	...	181	1.7	191	2.5	161	12.5
...	...	193	13.5	193	1.4	162	2.3
...	...	194	1.3	195	1.2	163	1.7
...	...	208M+	21.6	207	1.4	164	1.1
...	...	209	2.4	223	6.3	177	3.6
...	...	210	0.4	238M+	13.7	191	3.3
...	...	...	...	239	1.6	192M+	17.9
...	...	...	...	240	0.3	193	2.3
...	...	...	...	...	...	194	0.5

3.66 and 3.67 δ, and the aromatic protons as a singlet at 6.72 δ. The vinyl protons formed an ABC spin system, and that portion of the spectrum resembled the pattern for eugenol and safrol (10). The mass spectrum of methyleugenol (Fig. 3 and Table IV) showed that the parent peak, $M^+ = 178$, was the base peak. Several schemes are suggested to explain some of the peaks observed (Scheme I). Loss of a CH_3 radical produced the peak at *m/e* 163 (confirmed by a metastable ion at 149.2) which might be expected to lose CO to give a fragment (corresponding metastable peak at 179). The peak at *m/e* 184 (M-27) indicated the loss of a vinyl radical (4%), and *m/e* 177 (M-31) also appeared in 15% which is considerably lower compared to that of methyleugenol (25%). The successive loss of a methyl group and carbon monoxide, *m/e* 165 (M-15-28), was confirmed from the metastable peak at 141. A further loss of a methyl radical

[2] It was reported that the mass spectra of *o*- and *p*-dimethoxybenzenes showed peaks at *m/e* 123 (M-15) and *m/e* 95 (N-15-28) due to the successive loss of a CH_3 radical and then CO (*Reference 17*, p. 179).



Scheme I

from 165 produces a peak *m/e* 150, and the corresponding metastable peak at 136.3 was also observed. The initial fragmentation of elemicin may proceed by an analogous path to that of methyleugenol.

A minute quantity of 2,3,4,5-tetramethoxyallylbenzene was isolated whose NMR and mass spectra (Fig. 3, Table IV) were identical to an authentic sample obtained from parsley (16). Its NMR spectrum ($CDCl_3$) showed only three OCH_3 singlets at 3.80, 3.87, and 3.93δ, (in the ratio of 2:1:1) and the aromatic proton at 6.48δ and the pattern usually associated with the allyl group.

The only other aromatic compound which was isolated at this time was methylconiferaldehyde (3,4-dimethoxycinnamaldehyde), which was identical to an authentic sample. Its NMR spectrum ($CDCl_3$) showed the OCH_3 protons as a singlet at 3.80δ, the aldehyde proton at 9.6δ (J = 8 c.p.s.), and the rest of the spectrum similar to that of cinnamaldehyde. Its mass spectrum (Fig. 4, Table IV) showed the base peak at M^+ = 192, and the successive loss of a CH_3 radical (M-15, *m/e* 177, metastable peak at 163.1) followed by the loss of CO (M-15-28, *m/e* 149 metastable peak at 125.4). The successive loss of a OCH_3 radical (M-31, *m/e* 161, metastable at 135) and then CO (M-31-28, *m/e* 133, metastable at 109.1) was also confirmed, this CO stemming probably from the aldehyde side chain, a behavior observed previously in aromatic aldehydes (17).

Safrole could not be detected in any of the fractions collected, nor could there be found any propenylbenzenes. The latter would have shown up readily in the NMR spectra, since the CH_3 doublet of the CH_3CH=CH—group would have been readily discernible.

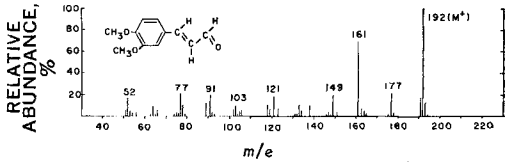


Fig. 4—The mass spectrum of 2,4-dimethoxycinnamaldehyde.

Aristolone—This sesquiterpene ketone was isolated from the steam volatile oil and the nonvolatile residue. It was shown to be identical to a sample previously isolated from *Aristolochia debilis* (18) and to an enantiomer, ferulone, obtained after air-oxidation of ferulene which was found in *Ferula communis* (19).

The structure and stereochemistry of aristolone had been established and the ketone shown to possess a cyclopropane ring (18). Although the cyclopropane protons were identified clearly in the NMR spectra of a number of hydrocarbons possessing the aristolane skeleton (19, 20), these protons were not clearly discernible in our 60-Mc. spectra of aristolone but were identified clearly in the 100-Mc. NMR spectrum[3] in C_6D_6 (Fig. 5, see under *Experimental*).

The mass spectrum of aristolone (Fig. 6) showed the molecular ion peak to be the base peak at *m/e* 218. The major fragmentation peaks were those resulting from loss of 15 (CH_3), 29 (C_2H_5), 43 (C_3H_7), 57 (C_4H_9), 71 (C_5H_{11}), 85 (C_6H_{13}), and 99 (C_7H_{15}) from the molecular ion, the charge remain-

[3] Determined on a Varian HA-100 NMR spectrometer by LeRoy F. Johnson, Varian Associates, Inc., Palo Alto, Calif. The authors thank Mr. Johnson for obtaining this spectrum and also for performing the decoupling experiments.

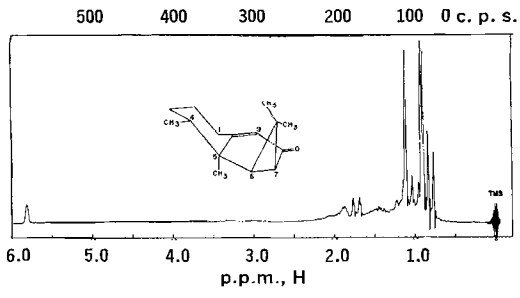


Fig. 5—The NMR spectrum of aristolone in C_6D_6 at 100 Mc.

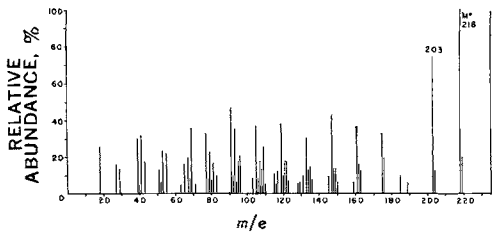


Fig. 6—The mass spectrum of aristolone.

ing with the oxygen-containing fragment. The corresponding metastable peaks for production of the seven fragments were also observed.

β-Sitosterol—This steroid was isolated from the steam-nonvolatile fraction *B* and identified by comparison with an authentic sample. This steroid is widely distributed in plants (21) and interestingly enough has just been found in *A. europeum* (22).

EXPERIMENTAL

Boiling and melting points are uncorrected. Mass spectra were obtained by means of the Hitachi RMU-6D mass spectrometer, ionizing voltage was 75 e.v., inlet temperature for the terpene derivatives being kept to a minimum. In recording the spectra, relative intensities of each fragment above 5% of the strongest line are shown except in the region of the molecular ion where all lines are included. NMR spectra were determined by means of the Varian A-60 spectrometer using tetramethylsilane (TMS) as internal reference, all signals being recorded in parts per million (δ) downfield from TMS. Infrared spectra were taken between salt plates or in $CHCl_3$ solution on a Perkin-Elmer model 337 spectrophotometer.

Gas Chromatography—Preparative GC was carried out by means of the Aerograph Autoprep model A-700 chromatograph using 20-ft. × 3/8-in. o.d. coiled aluminum columns packed with either 20% silicon gum rubber (SE-30) on Chromosorb W (40–60 mesh), or with 20% diethyleneglycol succinate (DEGS) on firebrick (40–60 mesh), both purchased from Wilkins Instrument and Research Co., Inc. All runs were nonlinearly temperature programmed at the "50" power setting, helium was used as the carrier gas, the flow rate was 200 ml./min. as measured at the outlet. For preparative runs, 0.4 to 0.6-ml. aliquots were injected repeatedly, the fractions trapped in 5-ml. glass traps, and cooled in dry ice and 2-propanol. After collection, the fractions were permitted to come to room temperature and reinjected in 1–5-μl. aliquots into a 150-ft. × 0.01-in. o.d. analytical column coated with castorwax (isothermal runs) in a Perkin-Elmer model 800 chromatograph, the flow of helium being 5 ml./min. as measured at the outlet.

Authentic Samples—The following samples were obtained commercially: β-myrcene (Aldrich Chemical Co.), α-pinene and dipentene (K and K Laboratories), geraniol and linalool (Eastman Chemicals), α-terpineol and linalyl acetate (Matheson Scientific Co.), bornyl acetate (Baker Chemical Co.). These were either distilled *in vacuo* or steam distilled and then purified by preparative GC (SE-30 column, Autoprep) under the general conditions described above and the required fraction collected. If the cut was not pure, it was then passed through a column of 30% FFAP on Chromosorb W column (Autoprep) until a pure fraction was obtained.

Methyleugenol was prepared from eugenol in a fashion described for the synthesis of anisole (23), b.p. 156–159° at 40 mm. [Lit. (24) b.p. 237–239°.] Isomethyleugenol was prepared by the isomerization of methyleugenol (10 Gm.) with hot saturated alcoholic KOH (10 ml.) instead of sodium (25) at 200° for 1 hr., b.p. 163–168° at 38 mm. [Lit. (26) b.p. 263°.]

3,4-Dimethoxycinnamaldehyde was prepared (27) from veratraldehyde and acetaldehyde and purified finally by GC, m.p. 84–85°. [Lit. (27) m.p. 82–83°.]

Isolation of Steam-Volatile Oil A—The plants grew in dense patches in shaded areas along the Des Plaines River in the Dam IV Forest Preserve (about 25 miles northwest of Chicago, Ill.) and were harvested (in small samples from each patch) during May 1960 and 1961.[4] Dry rhizomes were also purchased from the Meer Corp. in 1964.[5]

Eight kilograms of dried milled rhizomes were extracted continuously with hot pentane (b.p. 35–36°) for 1 week and the solvent removed (Vigreux column) on the steam bath. The residue (210 Gm., 2.6%) was steam distilled (5 hr.), and the distillate extracted with pentane. Evaporation of the solvent yielded 100 Gm. of a fragrant light yellow oil (*A*) which was examined immediately by GC. (Fig. 1, Table I).

Column Chromatography of Steam-Volatile Oil A—The oil *A* (50 Gm.) was placed on neutral alumina (750 Gm., Woelm, grade I) and eluted with various solvents until no further oil was obtained with a particular solvent (usually about 1.5 L.). Pentane was distilled through a Vigreux column at 760 mm., but other solvents were removed *in vacuo* using a rotatory flash evaporator at the lowest possible temperature, and the residue weighed. Each fraction then was subjected to preparative

[4] The authors thank Mr. Roland F. Eisenbeis, Superintendent of Conservation, Forest Preserve District, for permission to harvest the plants. The authors thank Mr. Floyd A. Swink, Morton Arboretum, Lisle, Ill., who identified the specimens as *Asarum canadense*, the plants usually found in this area, and who pointed out to us that *Asarum canadense* is polymorphic, *sensu latu*. A detailed discussion of the minute differences distinguishing four varieties of *A. canadense* L. is given by J. Steyermark ("Flora of Missouri," Iowa State University Press, Ames, Iowa, 1963, p. 572).

[5] Dr. William A. Meer reported to us that this material was collected during the spring and summer in northwestern Ohio and met the requirements of the N.F. VII.

GC under the conditions described in Fig. 1. The results of these separations are summarized in Table II.

Oxidation of Elemicin—A suspension of elemicin (0.1 Gm.) in aqueous $KMnO_4$ (0.5 Gm. in 10 ml.) was stirred and boiled under reflux for 1 hr. On cooling, $NaHSO_3$ (0.4 Gm.) was added, the mixture acidified with concentrated HCl, and extracted with $CHCl_3$. There was isolated 3,4,5-trimethoxybenzoic acid (0.05 Gm., 50%), m.p. 165°, M^+ 212, and whose infrared spectrum was identical to an authentic sample, m.p. 168° (Eastman Chemicals).

Chromatography of Linalyl Acetate—The ester (1 Gm.) was placed on neutral alumina (20 Gm.), and the following components were eluted and their identity proved by GC (enrichment method): β-myrcene (0.2 Gm., by pentane), unchanged ester (0.2 Gm., by ether), and linalool (0.1 Gm., by chloroform–ethanol, 9:1).

Separation of Steam-Nonvolatile Oil B—The steam-nonvolatile residue (after 5-hr. distillation) was taken up in pentane and the solvent removed. The residual oil (50 Gm.) was placed on basic alumina (750 Gm., Alcoa grade F-20) and eluted by each of the solvents shown in Table V. Some of the fractions then were distilled *in vacuo* and the distillates examined by GC.

Aristolone—The ketone was conveniently isolated from fraction *B*-VI. Short-path distillation of a portion of this fraction (1.5 Gm.) between 100–105° at 0.15 mm. afforded a viscous oil which slowly solidified. Sublimation of this solid at 100–110° at 0.075 mm. yielded the ketone (0.4 Gm.), m.p. 100–101° [lit. (18) m.p. 100–101°], undepressed on admixture with an authentic sample (18). $[\alpha]_D^{25}$ −321.3° (c, 1.5, $CHCl_3$), ν C=O in $CHCl_3$ 1640 $cm.^{-1}$. [Lit. (18) ν C=O in $CHCl_3$ 1658 $cm.^{-1}$.] $\lambda_{max.}^{EtOH}$ 234 (log ϵ 4.18), 310 mμ (log ϵ 2.15). [Lit. (18) $\lambda_{max.}^{EtOH}$ 235 (log ϵ 4.11), 310 mμ (log ϵ 2.07).]

The chemical shifts of aristolone in CCl_4 did not quite agree with those reported (20) (see Table VI), and in particular the cyclopropane resonances were not clearly discernible. However, the 100 Mc. spectrum[4] in C_6D_6 (Fig. 5) showed the resonances of H-6 and H-7 as two doublets, and, as expected, H-7 was considerably more shielded due to the inductive and anisotropic effect of the C=O group. The chemical shifts of H-7 and H-6 compared well with those observed for the α- and β-protons of cyclopropyl ketones (28, 29), and the magnitude of the coupling constant was of the order expected for adjacent *cis* cyclopropane protons (29).

The signal arising from the cyclopropane proton adjacent to the C=O group (H-7) showed fine splitting (J = 1.1 c.p.s.) which was traced to spin–spin interaction with the vinyl proton. This coupling through the carbonyl group (30) was proved by irradiating the vinyl signal (H-9) at 5.77 δ (in C_6D_6) while sweeping the rest of the spectrum which changed the signal at 1.72 δ to a simple doublet. Irradiation of the signal at 1.72 δ (H-7) sharpened the unresolved multiplet at 5.77 δ, and turned the doublet at 0.99 δ to a singlet, thus establishing the chemical shift of H-6. The only other change which was observed by irradiating at 1.72 δ was the collapse of the methyl doublet at 0.80 δ to an ill-defined broad signal, which suggests that the chemical shift of the methine proton at C-4 was in the vicinity of 1.72 δ. A considerable solvent effect

TABLE VI—NMR SPECTRAL DATA FOR ARISTOLONE

Protons and Coupling Constants Involved	C_6D_6 100 Mc.	C_6H_6 60 Mc.	$CDCl_3$ 60 Mc.	CCl_4 60 Mc.	CCl_4[a]
CH_3 Doublet at C-4	0.80	0.81	1.10	1.07	0.93
$J_{CH_3,H-4}$	(6.7)	(6.5)	(6.2)	(6.0)	(6)
CH_3 Singlets at C-5, C-11	0.90	0.90	1.20	1.20	1.21
	0.92	0.92	1.20	1.20	1.28
	1.12	1.12	1.25	1.24	—
H-7	0.99	[b]	1.38	(1.30)[c]	1.38
H-6	1.72	1.72	1.72	1.60	1.74
$(J_{6,7})$	(8.5)	(8.4)	(8.2)	(8.0)	(8)
$(J_{7,9})$	(1.1)	(1.1)	(1.2)	(1.4)	(1)
H-9	5.77	5.78	5.72	5.60	5.73

[a] Data from *Reference 20.* [b] Doublet not clearly observable. [c] This chemical shift was calculated on the assumption that in this solvent H-6 and H-7 were observed as an AB quartet. However, the fourth line necessary to compute this value would be hidden underneath the CH_3 singlets at 1.22 δ.

TABLE V—CHROMATOGRAPHY OF OIL *B*

Fraction	Eluting Solvent	Yield, Gm.	Comments
B-I	Pentane	9.4	Fractions, b.p. 70–135° at 0.4 mm. (5 Gm.), contained varying quantities of linalool, bornyl acetate, methyleugenol, and elemicin; fractions, b.p. 130–170° at 0.1 mm. (4.0 Gm.), could not be separated and identified.
B-II	Pentane–benzene (9:1)	6.7	Methyleugenol (99%)
B-III	Benzene	12	Fractions, b.p. 80–105° at 0.1 mm. (3 Gm.), consisted of varying mixtures of methyleugenol, elemicin, and 2,3,4,5-tetramethoxyallylbenzene; b.p. 106–125° at 0.1 mm. (1 Gm.) contained aristolone in addition to the ethers.
B-IV	Benzene–ether (9:1)	5.5	Aristolone (see under *Aristolone*)
B-V	Ether	0.5	Not examined further.
B-VI	Ether–chloroform (9:1)	2.0	β-Sitosterol (see under *β-Sitosterol*)
B-VII	Chloroform	2.0	β-Sitosterol (see under *β-Sitosterol*)
B-VIII	Chloroform–ethanol (9:1)	3.0	Linalool (88%)
B-IX	Ethanol	—	

was noted for the chemical shifts of one of the cyclopropane protons (H-7) and that of the CH_3 resonances in benzene and chlorinated solvents. (Table VI). These shifts are attributed to benzene–carbonyl solvent complexes (31) but in the absence of model compounds, no assignment for the chemical shifts of different CH_3 groups is made at present.

Anal.[6]—Calcd. for $C_{15}H_{22}O$: C, 82.51; H, 10.15. Found: C, 82.26; H, 10.29.

Its 2,4-dinitrophenylhydrazone crystallized from ethanol, m.p. 165°. [Lit. (18) m.p. 169–170°.]

Anal.[7]—Calcd. for $C_{21}H_{26}N_4O_4$: N, 14.06. Found: N, 13.90.

β-Sitosterol—Rechromatography of fraction *B*-VI (2 Gm.) on basic alumina (50 Gm.) gave in the ether–chloroform (9:1) eluent a light yellow solid (1 Gm.) which was extracted with hot pentane. The pentane solution was evaporated and the residue recrystallized from ethanol to give the product (0.5 Gm.), m.p. 137°. [Lit. (32) m.p. 137°.] It was undepressed by an authentic sample. $[\alpha]_D^{24}$ −37.07° (c, 0.47 in $CHCl_3$). [Lit. (32) $[\alpha]_D^{25}$ −37.0 ($CHCl_3$).] ν OH in $CHCl_3$ at 3600 cm.$^{-1}$. Its NMR spectrum showed one vinyl proton at 5.38 δ and the >CH—O— proton as a multiplet at 3.50 δ, and the rest of the spectrum was like that published previously (33). Its mass spectrum is reproduced in Fig. 7 and showed *m/e* 414.

Anal.[6]—Calcd. for $C_{29}H_{50}O$: C, 83.98; H, 12.15. Found: C, 83.83; H, 12.26.

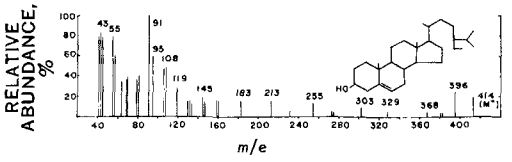


Fig. 7—The mass spectrum of β-sitosterol.

β-Sitosterol Benzoate—A solution of β-sitosterol (0.2 Gm.) in pyridine (1.5 ml.) was treated with benzoyl chloride (0.2 Gm.) and heated over a free flame for 1 min. The solution was poured into ice water, the solid filtered, and washed with 5% K_2CO_3 solution. After crystallization, the solid melted at 143°. [Lit. (32) m.p. 146°.] ν C=O in $CHCl_3$ at 1740 cm.$^{-1}$, M^+ at *m/e* 518.

[6] Microanalysis by Micro-Tech Laboratories, Skokie, Ill.

[7] Microanalysis by Mr. Leo Horner, Department of Chemistry, University of Illinois at the Medical Center, using a Coleman nitrogen analyzer, model 29.

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