chromatograph was equipped with a 4.8 × 610 mm copper column packed with Carbowax 20M on a Chromosorb W, 60–80 mesh support and a FID and operated at 120°. All steps were repeated on triplicate samples and resulting data were averaged.

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IDENTIFICATION OF VOLATILE CONSTITUENTS OF *SASSAFRAS ALBIDUM* ROOT OIL*

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Key Word Index—*Sassafras albidum*; Lauraceae; root oil; monoterpenes; sesquiterpenes; allylbenzenes; propenylbenzenes; aromatic aldehydes.

Abstract—Sassafras root bark oil was examined by GC–MS for the possible natural occurrence of 1′-hydroxysafrole, a potent hepatocarcinogenic mammalian metabolite of safrole. Six monoterpenes, 2 sesquiterpenes, 6 allylbenzenes, 2 propenylbenzenes, 2 acroleins and 1 benzaldehyde derivative were identified. Eleven out of these 19 sassafras constituents are reported for the first time. However, 1′-hydroxysafrole was not detected.

INTRODUCTION

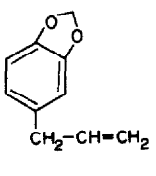
Safrole (**1**) is the major constituent (about 80%) of sassafras oil obtained from the root bark of *Sassafras albidum* (Nutall) Nees (Lauraceae) by steam distillation. Prior to 1960, both sassafras oil and safrole were used extensively as flavors in confections, soft drinks such as root beer, and in pharmaceutical preparation. In 1960, the United States Food and Drug Administration banned the use of safrole as a food additive when it was found to be hepatocarcinogenic in the rat [1–4].

Recent metabolism studies showed that 1′-hydroxysafrole (**2**) was a proximate carcinogenic metabolite of safrole in several experimental animals [5]. The finding that the hydroxy metabolite (**2**) was a more potent hepatocarcinogen than safrole [6] prompted the present investigation of sassafras root bark for the possible natural occurrence of (**2**) and related compounds. A detailed phytochemical study of this plant material was also of interest in our current program of isolation, structure elucidation and bioassay of potentially carcinogenic natural products [7–10].

RESULTS AND DISCUSSION

Table 1 lists the nineteen compounds identified by GC–MS in the sassafras root bark oil fractions A and B. These include six monoterpenes (**3–8**), two sesquiterpenes (**10** and **11**), six allylbenzenes (**1**, **12**, **13**, **15**, **17**, and **20**), two propenylbenzenes (**9** and **16**), two acrolein derivatives (**14** and **18**), and one benzaldehyde derivative (**19**). Among the nineteen compounds reported here, compounds **1**, **3–6**, **12**, **14**, and **18** were identified earlier in the sassafras root [20,23,24].

Safrole (**1**) was the major volatile constituent (about 90%) in oil A, while oil B contained 5-methoxyeugenol (**17**, 30%), asaron (**16**, 18%), piperonylacrolein (**18**, 11%), coniferaldehyde (**14**, 7%), safrole (**1**, 6%), and camphor



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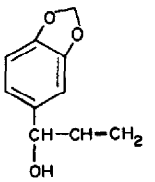

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* Part 5 in the series "Potential carcinogens"; for Part 4 see ref. [10].

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Table 1. Volatile constituents identified in the sassafras root bark oil by GC–MS

Compound number	Compound	Reference to MS data	Elution temperature (°C)	Molecular ion (*m/e*)	Identified in Oil A	Oil B
3	α-Pinene	11	75	136	+	−
4	Camphor	12	76	152	+	+
5	α-Phellandrene	11	77	136	+	+
6	β-Phellandrene	11	78	136	+	−
7	*l*-Menthone	12	80	154	+	−
8	Thujone	12	81	152	+	−
9	Anethole	13	90	148	+	−
10	Copaene	14	105	204	+	−
1	Safrole	15	110	162	+	+
11	Caryophyllene	15	115	204	+	−
12	Eugenol	16	120	164	+	+
13	Elemicin	17	125	208	+	+
14	Coniferaldehyde*	—	130	178	+	+
15	Myristicin	18	145	192	+	+
16	Asaron*	—	150	208	+	+
17	5-Methoxyeugenol*	19	155	194	+	+
18	Piperonylacrolein	20	162	176	+	+
19	Syringaldehyde	21	165	182	−	+
20	Apiol	22	190	222	−	+

Reference 1′-hydroxysafrole eluted at 160° and exhibited a molecular ion at *m/e* 178 [5]. *Identification confirmed by isolation of the product.

(**4**, 5%). The remaining constituents reported in Table 1 were present in trace quantities (less than 1%).

Recently, 1′-hydroxysafrole (**2**) was reported as a potent hepatocarcinogenic mammalian metabolite of safrole [5,6]. The present GC–MS examination of the sassafras root oil failed to detect 1′-hydroxysafrole in the sassafras oil fractions A and B. However, this does not rule out the possibility that **2** may be formed in the plant as a transitory intermediate which may not be easy to isolate or detect since it contains a very reactive hydroxyl group located on an allylic and benzylic carbon atom. It is conceivable to envision **2** as an intermediate in the biosynthesis of lignans containing oxygen on the benzylic carbon, such as sesamin which occurs in the sassafras plant [20].

Among the allyl and propenylbenzene derivatives reported here as minor constituents of sassafras root bark, eugenol (**12**) and anethole (**9**) are known to be weak hepatotoxins [25,26]. Thus, safrole appears to be the primary hepatocarcinogenic volatile constituent of the sassafras root bark oil.

EXPERIMENTAL

Plant material. The powdered root bark of *Sassafras albidum* was supplied by I. D. Auld, Jr., 667 Ferry Street, Mt. Pleasant, South Carolina.

Solvent extraction of the plant material. Powdered root bark of *Sassafras albidum* (150 g) was extracted with 3 l. of petrol (bp 35–60°) at room temp. The petrol extract was evaporated to dryness *in vacuo* to yield 5.45 g of an oily residue. Oily residue was extracted 4 × with 50 ml 70% aq. MeOH and each extract was washed with 50 ml petrol. The MeOH extracts were pooled and MeOH removed under red. pres. The resulting aqueous concentrate was extracted with CH_2Cl_2 (3 × 50 ml). Evaporation of the CH_2Cl_2 extract to dryness afforded 439 mg of oily residue (Oil A). The petrol washings and the 70% aq. MeOH-insoluble residue were combined, and petrol removed *in vacuo* to yield 4.87 g of oily residue (Oil B).

GC–MS. GC–MS employed a 6 ft. glass column containing 1% OV-17 on 80–100 mesh Supelcoport. Helium was used as the carrier gas at a flow rate of 15 ml/min. A 2–5 mg quantity of the oil samples were dissolved in 0.2 ml dry Et_2O or MeOH and a 2 μl aliquot was injected into the GLC column. The column temp. was programmed from 70°–200° at a rate of 4°/min. A total ion current detector was used to monitor the chromatographic separation of constituents. All MS were obtained at 70 ev electron beam voltage. The general procedure employed for the GC–MS identification of the unknowns was described earlier [27,28]. The NIH computerized mass spectral search system [29] and the computer assembled molecular index of the Merck Index for mass spectrometry [30] were used.

Quantitation of sassafras constituents was carried out by peak area measurements from the GLC profiles recorded during GC–MS.

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15-DEOXYGOYAZENSOLIDE, A NEW HELIANGOLIDE FROM *VANILLOSMOPSIS ERYTHROPAPPA**

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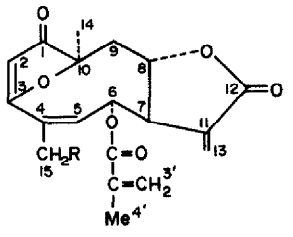
Key Word Index—*Vanillosmopsis erythropappa*; Compositae; heliangolide; sesquiterpene lactone.

INTRODUCTION

In an earlier article [1] the isolation of eremanthin [2] and costunolide from the schistosomicidal wood oil of *Vanillosmopsis erythropappa* Sch. Bip, (Vernonieae, Vernoninae) was reported [3]. We now describe isolation and structure determination of 15-deoxygoyazensolide (**1a**) from a hexane extract of the herbaceous parts of this plant.



1a R = H
b R = OH

DISCUSSION

15-Deoxygoyazensolide (**1a**), $C_{19}H_{20}O_6$ (high resolution MS), mp 132–4°, $[\alpha]_{Hg}^{24}$ −38°, was a γ-lactone with a conjugated exocyclic methylene group (IR band 1770 and 1650 cm^{-1}, strong UV end absorption, sharp doublets at 6.21 and 5.45 ppm in the NMR spectrum) and a methacrylic ester function (IR bands at 1710 and 1640 cm^{-1}, broad vinyl methyl signal at 1.82 ppm and two multiplets at 6.01 and 5.54 ppm, significant MS peak at *m/e* M–C_4H_5O).

The NMR spectrum of the new lactone (see Table 1) was essentially superimposable on that of goyazensolide (**1b**) from *Eremanthus goyazensis* Sch. Bip. [5] except for the resonances of the C-15 hydroxymethylene protons of **1b**, which were replaced by a narrowly-split vinyl methyl triplet at 2.06 allylically coupled to a vinyl proton

Table 1. 270 MHz ^{1}H-NMR spectrum of **3a***

H-2	5·70	H-13a	6·21*d* (3·3)
H-5	6·01*m*	H-13b	5·45*d* (3·0)
H-6	5·0*m* (2·0, 2·5)	H-14	1·51†
H-7	3.72*m* (2·5, 2·5, 3·3, 3·0)	H-15	2·06*t* (2·0)†
H-8	4·63*td* (2·5, 11 5)	H-3^{1}a	6·01*m* (1)
H-9a	2 34*dd* (14,12)	H-3^{1}b	5·54*m* (1·5)
H-9b	2·29*dd* (2·0, 14·0).	H-4^1	1·82*m*†

* Run in $CDCl_3$ with TMS as internal standard. Values are in ppm. Multiplicities are given by the usual symbols. Unmarked signals are singlets. Figures in parentheses are coupling constants in Hz. † Intensity three protons.

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