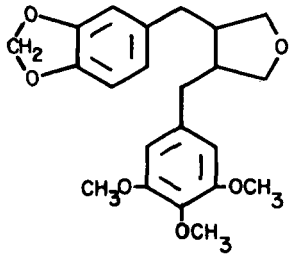
Antitumor Agents from *Bursera microphylla* (*Burseraceae*) II: Isolation of a New Lignan—Burseran

J. R. COLE, E. BIANCHI, and E. R. TRUMBULL*

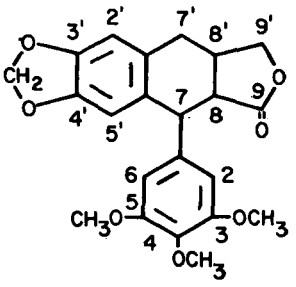
Abstract □ *Bursera microphylla* (*Burseraceae*) A. Gray has yielded a second component which has demonstrated activity in the cell culture test system of the Cancer Chemotherapy National Service Center (CCNSC). On the basis of NMR, mass, and IR spectrometry, the structure of a new lignan, 3-(3,4-methylenedioxybenzyl)-4-(3′,4′,5′-trimethoxybenzyl) tetrahydrofuran (named burseran), is proposed.

Keyphrases □ Antitumor agents—*Bursera microphylla* □ Burseran—structure identification □ Mass spectroscopy—structure □ IR spectrophotometry—structure □ NMR spectroscopy—structure

In the previous paper in this series (1) the authors have reported the presence of deoxypodophyllotoxin (III) which has been shown to demonstrate tumor-inhibitory properties. In the continuing search for antitumor agents from plants, it was observed that *Bursera microphylla* yielded a second component (II) which also demonstrated tumor-inhibitory properties (2.6×10^{-2} mcg./ml.) against the human epidermoid carcinoma of the nasopharynx test system (cell culture) of the CCNSC (9KB). Activity is defined as $ED_{50} \leqq 10$ mcg./ml. for plant extracts. Results reexpressed as the dose that inhibits growth to 50% of control growth 3 days after drug addition (2).



II



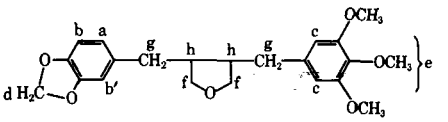
III

Since the yield of this compound from the plant was so small, it was decided to elucidate its structure and then prepare larger quantities by synthetic methods. The next report in this series will describe the total synthesis of this component.

There are a great number of structural similarities between the burseran and the previously isolated deoxypodophyllotoxin. The employment of spectrometric methods for identification purposes has been used to show the relationships between the two compounds. Burseran lacked the lactone band that appeared in the IR spectra of deoxypodophyllotoxin at 5.65 μ. This fact was supported by the NMR spectra lacking the signal at 4.65 p.p.m. which corresponded to the position 9′ of the lactone ring of deoxypodophyllotoxin. The molecular weight and elemental analyses indicated that burseran differed from deoxypodophyllotoxin only in the loss of an oxygen atom and with an increase of four hydrogen atoms. It appeared that the carbonyl group of deoxypodophyllotoxin, therefore, had been replaced by a methylene group and since an additional aromatic proton was present in burseran, this suggested that the bond between position 7 and the methylenedioxy substituted aromatic ring was not present. It would also appear that the structure for Compound II is reasonable on biogenetic grounds when compared to deoxypodophyllotoxin.

On the basis of mass spectrometry and elemental analyses, the molecular formula of burseran is $C_{22}H_{26}O_6$.

Table I—Interpretation of the NMR Spectra of Burseran[a]



Proton	Position (δ)	Form	Intensity
a	6.52 or 6.34	Doublet	1
b	6.52 or 6.34	Doublet	1
b′	6.49	Singlet	1
c	6.2	Singlet	2
d	5.72	Singlet	2
e	3.78	Singlet[b]	9
f	4.0 to 3.3	Complex	4
g	2.5	Asymmetrical doublet	4
h	2.3 to 2.0	Complex	2

[a] Compound dissolved in deuterochloroform; spectra run on Varian model A-60 and HA-100. [b] Split to Doublet 6 and 3 by addition of benzene.

EXPERIMENTAL

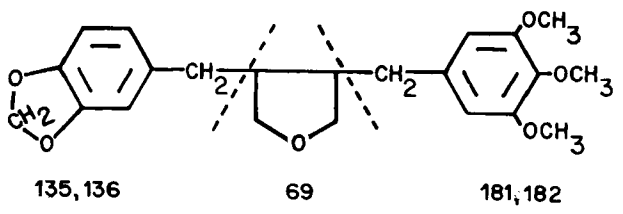
Isolation—The extraction procedure has been described previously (1). The active fraction of the plant was obtained from the alumina column by elution with benzene. It contained Compounds I (unknown and in very small quantity), II (now characterized as burseran), III (deoxypodophyllotoxin), and IV (β-sitosterol).

Burseran is an oily substance which has defied all attempts at crystallization. The synthetic product, which will be described in a later publication, verifies the oily character. The separation of Compound III from Compound II has already been described (1). However, this was not a satisfactory method for the isolation of Compound II.

A slight modification in the previous procedure was made to facilitate the isolation of the compound in as pure a form as possible. This involved the employment of the alumina column plus a diatomaceous earth[1]-dimethylformamide column. The column was prepared with 400 g. of diatomaceous earth, previously screened

[1] Celite, Johns-Manville, New York, N. Y.

Table II—Interpretation of Mass Spectrum of Burseran[a]



Mass[b]	Ion
387	P + 1
386	P
181, 182	$C_{10}H_{13}O_3$ and $C_{10}H_{14}O_3$
167	Loss of CH_3 from 182 ion
151	Loss of OCH_3 from 182 ion
135, 136	$C_8H_7O_2$ and $C_8H_8O_2$
77	C_6H_5
69	C_4H_5O

[a] Mass spectrometry was done utilizing a Perkin-Elmer Hitachi mass spectrometer model RMU6E. The molecular formula was then calculated as $C_{22}H_{26}O_6$. *Anal.*—Calcd. for C, 68.39; H, 6.74; CH_3O, 24.09. Found: C, 68.80; H, 6.90, CH_3O, 22.75. Elemental analyses made by Huffman Laboratories, Wheatridge, Col. [b] Tentative assignments.

to 100–200 mesh, washed with two portions of ethanol, dried, and then washed with two portions of acetone (1 l. of each solvent was used). It was dried in the air and then in an oven at 110° for 18 hr. An additional 200 ml. of formamide (99%) was added and it was shaken mechanically. This mixture was then used in the preparation of the diatomaceous earth column. The column, 50 mm. in diameter, was filled to approximately the 61-cm. (24-in.) level with diatomaceous earth-dimethylformamide (300 g.). It was packed under 20-lb. pressure N_2.

The fractions obtained from the alumina column in which Compound II appeared to be relatively pure were rechromatographed through the diatomaceous earth-dimethylformamide column. Elution of this column with hexane was employed to produce a fraction containing an almost pure sample of the compound. This elution was very slow, but very effective. The material was then applied to TLC utilizing Silica Gel G[2] since there were still some impurities present. It was necessary to rerun this TLC analysis five times in order to completely remove all signs of impurity. The solvent system employed for this procedure was dichloromethane–benzene–ethyl acetate (12:24:3).

Tables I and II describe the interpretation of the various spectra. The IR spectra of the compound is presented in Fig. 1.[3]

[2] Merck and Co., East Rutherford, N. J.
[3] Run on a Perkin-Elmer Infracord spectrophotometer model No. 137.

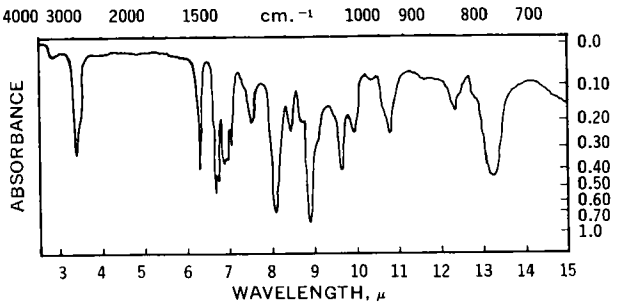


Figure 1—*Infrared curve of 3-(3,4-methylenedioxybenzyl)-4-(3′,4′,5′-trimethoxybenzyl) tetrahydrofuran (burseran).*

SUMMARY

Bursera microphylla has yielded a second component which has shown tumor-inhibitory properties against the 9KB (cell culture) test system of the CCNSC. On the basis of mass, NMR, IR, and elemental analyses, it was found to be a new compound of oily character having a molecular weight of $C_{22}H_{26}O_6$. Its structure has been proposed as 3-(3,4-methylenedioxybenzyl)-4-(3,′4′,5′-trimethoxybenzyl) tetrahydrofuran and the compound has been named burseran. The third report in this series will describe the total synthesis.

REFERENCES

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(2) "Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems," Cancer Chemotherapy Reports No. 25, Cancer Chemotherapy National Service Center, U. S. Department of Health, Education, and Welfare, Washington, D. C., Dec. 1962.

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Antitumor Agents from *Bursera microphylla* (*Burseraceae*) III: Synthesis of Burseran

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Abstract □ Burseran, 3-(3,4-methylenedioxybenzyl)-4-(3′,4′,5′-trimethoxybenzyl) tetrahydrofuran, has been synthesized in order to prove its proposed structure.

Keyphrases □ Antitumor agents—*Bursera microphylla* □ Burseran—synthesis □ NMR spectroscopy—identity □ UV spectrophotometry—identity □ IR spectrophotometry—identity □ GLC—identity □ Mass spectroscopy—identity

In a prior publication (1) the authors have reported the isolation of a new lignan, 3-(3,4-methylenedioxybenzyl)-4-(3′,4′,5′-trimethoxybenzyl) tetrahydrofuran, which has been named burseran. Since the proposed structure of the compound was determined mainly by spectral evidence, it was necessary to prove this structure either by means of degradative reactions or by a total synthesis. Because of the problems expected in proof of structure by means of degradation and/or ring closure, total synthesis appeared to be the preferred approach.