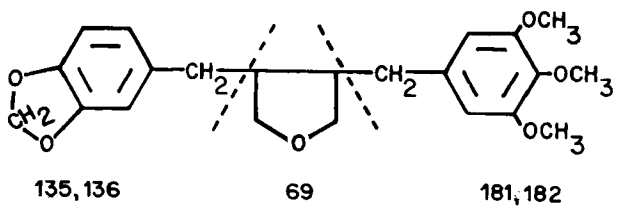
Table II—Interpretation of Mass Spectrum of Burseran[a]



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.

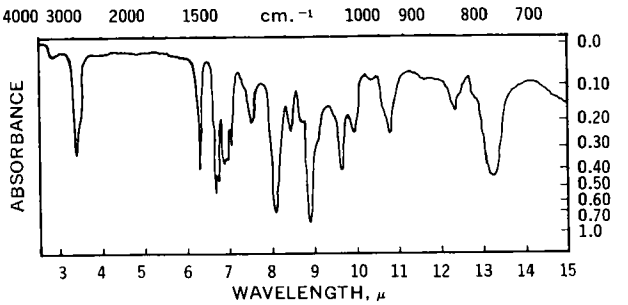


Figure 1—*Infrared curve of 3-(3,4-methylenedioxybenzyl)-4-(3′,4′,5′-trimethoxybenzyl) tetrahydrofuran (burseran).*

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.

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

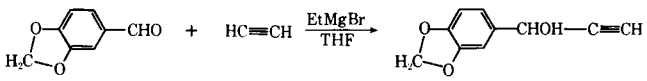
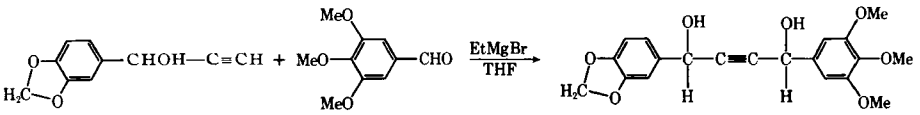
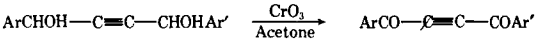
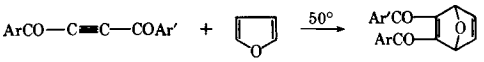
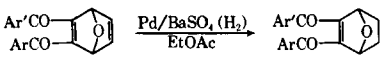
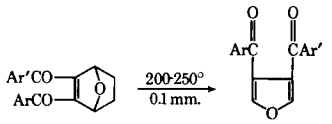
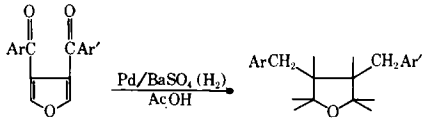
E. R. TRUMBULL* and J. R. COLE

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.

Table I—Synthesis of 3-(3,4-Methylenedioxybenzyl)-4-(3′,4′,5′-trimethoxybenzyl)tetrahydrofuran (Burseran)

Reaction No.	Reaction	Yield, %
1		80
2		78
3		54
4		quant.
5		85
6		85
7		27

The synthesis of the proposed structure is complicated by the fact that the constituents in the aromatic nuclei are not identical. The synthetic approach adopted was based upon the Alder-Rickert synthesis of furan-3,4-dicarboxylic acid (2). This method depends on the fact that the Diels-Alder adduct of furan and acetylenedicarboxylic ester can be selectively hydrogenated at the isolated double bond and that pyrolysis of the dihydro adduct will yield ethylene and a substituted furan by reversal of the Diels-Alder addition reaction. The steps of the synthesis are outlined in Table I.

The product isolated from this synthesis is a mixture of the *cis* and *trans* isomers of burseran, since the NMR[1] spectra, paper chromatography, and gas chromatography all show that two similar compounds are present although the mass spectra[2] indicate only one parent peak, *i.e.*, one molecular weight. The IR[3] spectra and UV[4] spectra and carbon-hydrogen analyses[5] of the natural product are identical to those of the synthetic mixture. It would appear from the gas chromatographic analysis that the natural product is identical with the first material of the synthetic mixture eluted from the column as the retention times are identical.

Burseran is now being prepared in larger quantities in order to test the material for biological activity.

EXPERIMENTAL

α-Ethynyl-piperonyl Alcohol—The acetylenic carbinol was prepared in 80% yield by treatment of ethynyl magnesium bromide with piperonal (3). The product was purified by chromatography on a silicic acid–diatomaceous earth[6] column from which it was eluted with chloroform. The appropriate fractions were distilled under reduced pressure yielding a colorless oil, b.p. 105–110° at 0.05 mm., which solidified, m.p. 34–36° [lit. (4) m.p. 35–36°].

1-(Methylenedioxyphenyl-4-(3′,4′,5′-trimethoxyphenyl)-1,4-butynediol—The acetylenic carbinol prepared above was converted to the diol by a modification of the procedure described in the literature (5). A solution of ethyl magnesium bromide prepared from 2.0 g. (0.082 mole) of magnesium and 10.0 g. (0.092 mole) of ethyl bromide in 125 ml. of tetrahydrofuran was added over a 45-min. period to a solution of 6.2 g. (0.035 mole) of α-ethynylpiperonyl alcohol in 75 ml. of tetrahydrofuran. The reaction was run under nitrogen with stirring at room temperature and was allowed to stir for 20 min. after addition of the last portion of Grignard reagent. To this solution, a solution of 10.0 g. (0.036 mole) of 3,4,5-trimethoxybenzaldehyde in 50 ml. of tetrahydrofuran was added dropwise with stirring. When the addition was complete, the reaction mixture was kept at room temperature under nitrogen for 4 hr.

The reaction mixture was poured into 1 l. of water and adjusted to pH 7 by adding solid sodium bicarbonate, then dilute sulfuric acid. The neutral solution was extracted with four 200-ml. portions of chloroform and the extracts were dried over magnesium sulfate. Chloroform was distilled from the solution under reduced pressure with gentle warming until the pressure was reduced to 0.7 mm. The residue at this point was a thick yellow oil and weighed 19.0 g.

The crude product was chromatographed on a column containing 400 g. of silicic acid–diatomaceous earth (3:1). Impurities, largely unreacted aldehyde, were eluted from the column with chloroform and the acetylenic diol was eluted with chloroform containing 5% methanol. The product was a thick yellow-brown oil, weight 10.1 g. (77%), $\lambda_{max.}$ 282, log ε 3.57 (methanol).

Anal.—Calcd. for $C_{20}H_{20}O_7$ (372.4): C, 64.50; H, 5.41. Found: C, 64.49; H, 5.36. The IR and NMR spectra showed the features to be expected for this compound.

1-(3,4-Methylenedioxyphenyl)-4-(3′,4′,5′-trimethoxyphenyl)-1,4-butynedione—Oxidation of the diol to the corresponding di-

[1] Varian models A-60 and HA-100.
[2] Perkin-Elmer Hitachi mass spectrometer, model RMU6E.
[3] Perkin-Elmer Infracord, model 137.
[4] Beckman DBG spectrophotometer.
[5] Huffman Laboratories, Wheatridge, Colo.
[6] Celite, Johns-Manville, New York, N. Y.

ketone was accomplished by chromic acid in acetone (6). A solution of 3.0 g. (0.030 mole) of chromium trioxide in 2.4 ml. of concentrated sulfuric acid and 14 ml. of water was added in small portions to a solution of 7.5 g. (0.020 mole) of diol in 50 ml. of acetone. The reaction mixture was cooled in ice and stirred with a glass rod to keep the green precipitate that appeared in contact with the solution. The oxidizing agent was added over 30 min. and the reaction was allowed to stand an additional 0.5 hr. The mixture was then poured into 400 ml. of water and the yellow solid produced was collected by filtration. The crude product was purified by chromatography on a silicic acid–diatomaceous earth (3:1) column from which it was eluted with chloroform to give 4.2 g. (54%) of yellow solid melting at 159–161°. A sample for analysis was recrystallized from methanol. The diketone had $\lambda_{max.}$ 340 mμ, log ε 4.19 in ethanol, and showed the expected characteristics in the IR and NMR spectra.

Anal.—Calcd. for $C_{20}H_{16}O_7$ (368.3): C, 65.22; H, 4.38. Found: C, 65.17; H, 4.50.

Adduct of Furan and 1-(3,4-Methylenedioxyphenyl)-4-(3′,4′,5′-trimethoxyphenyl)-1,4-butynedione—A mixture of 2.3 g. of acetylenic dione and 10.0 ml. of furan was cooled and sealed in a glass tube. The tube was then placed in a furnace and heated at 50° for 20 hr. At the end of this period the solution was homogeneous and light yellow in color. The contents of the tube were transferred to a flask and evaporated to dryness, finally at 0.1 mm. The residue was a yellow foam, weight 2.75 g. (101%), $\lambda_{max.}$ 320 mμ, log ε 4.06, and $\lambda_{max.}$ 281 mμ, log ε 4.00. The adduct did not crystallize and was converted directly to the dihydro derivative.

Dihydro Adduct—The crude adduct, 2.75 g. was dissolved in 30 ml. of ethyl acetate and was added to a flask containing 0.48 g. of 5% palladium on barium sulfate. The solution absorbed 1.16 moles of hydrogen per mole of sample in 90 min. The reaction mixture was filtered to remove the catalyst and evaporated to dryness leaving a yellow solid. The crude material was recrystallized from ethanol to give 1.85 g. (67%) of product melting at 149–151°. A sample was recrystallized again from ethanol, m.p. 152–153°. The dihydro adduct had $\lambda_{max.}$ 320 mμ, log ε 4.12, and $\lambda_{max.}$ 277 mμ, log ε 4.11 in ethanol. The carbonyl band was found at 6.10 μ.

Anal.—Calcd. for $C_{24}H_{22}O_8$ (438.4): C, 65.75; H, 5.06. Found: C, 65.66; H, 5.15.

3-(3,4-Methylenedioxybenzoyl)-4-(3′,4′,5′-trimethoxybenzoyl) furan—A sample of the dihydro adduct weighing 0.384 g. (0.88 mmole) was placed in a length of 12-mm. tubing, sealed at one end, and creased about 10.16 cm. (4 in.) above the sealed end. The tube was placed in a furnace so that the part below the crease was heated while the tube above the crease was outside the furnace. The tube was evacuated to 0.05 mm. and the furnace temperature was raised slowly to 270°. A colorless oil collected in the cool part of the tube. When no further product could be seen condensing on the cool surface, the tube was removed from the furnace and the bottom part, containing some charred residue, was cut off. The product was rinsed from the upper portion of the tube. Products from several pyrolyses were combined and recrystallized from ethanol to give colorless needles, m.p. 153–155°, in 83% yield. A sample dried for analysis had a m.p. of 154–155°, $\lambda_{max.}$ 310 mμ, log ε 4.23 and $\lambda_{max.}$ 282 mμ, log ε 4.20 in ethanol.

Anal.—Calcd. for $C_{22}H_{18}O_8$ (410.3): C, 64.40; H, 4.42. Found: C, 64.24; H, 4.40.

3-(3,4-Methylenedioxybenzyl)-4-(3′,4′,5′-trimethoxybenzyl)-tetrahydrofuran—A solution of 1.02 g. (2.48 mmoles) of the diaroylfuran in 75 ml. of acetic acid was added to a flask containing 0.66 g. of 5% palladium on barium sulfate. The solution absorbed 6.1 moles of hydrogen per mole of sample in 5 hr. after which hydrogen uptake stopped. The reaction mixture was filtered to remove the catalyst and the acetic acid was removed by distillation at 40 mm. from a bath at 40°. Traces of acetic acid remaining were removed by codistillation with heptane. The residue was an oil, weight 1.06 g.

For purification, this material was chromatographed on a column of alumina III containing 200 g. of adsorbent. The tetrahydrofuran was eluted with benzene and tested for the presence of burseran by TLC. The combined weight of fractions consisting primarily of the desired material was 0.276 g. (28%). The purest fractions were further purified by chromatography on silica gel plates using a mixture of benzene, chloroform, and ethyl acetate in the ratio (5:3:1) as developing medium.

The IR spectrum of the synthetic product was identical to that of the natural product. However, gas chromatography using a 101.4-cm. (3-ft.) column containing 1% QF-1 on siliconized diatomaceous earth[7] at 240° and 10 p.s.i. indicated that the synthetic compound was a *cis-trans* mixture. The natural product when run under the same conditions has a retention time identical to that component of the synthetic mixture which is eluted first. This is probably the *trans* isomer which would be expected to have a lower dipole moment and, therefore, less interaction with the polar adsorbent.

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[7] Gas Chrom P, Applied Science Laboratories, Inc., State College, Pa.