Phytochemistry, 1964, Vol. 3. pp. 259 to 262. Pergamon Press Ltd. Printed in England

# THE MONOTERPENES OF LODGEPOLE PINE OLEORESIN

RICHARD H. SMITH

Pacific Southwest Forest and Range Experiment Station Forest Service,
U.S. Department of Agriculture, Berkeley, California

(*Received* 26 *August* 1963)

**Abstract**—A gas chromatographic analysis was made of the terpenes of lodgepole pine resin. Three terpenes not previously recorded were found in measurable quantities: limonene, 2·4%; sabinene, 2·1 %; and α-phellandrene, 0·7%. Heptane and an unidentified compound were also detected. Though a basic pattern of composition was observed, there was considerable variation between individual trees.

## INTRODUCTION

LODGEPOLE pine (*Pinus contorta* Dougl.) is one of the most widespread and morphologically variable North American pines. Critchfield[1] divided it into four subspecies on the basis of some of these variations. Mirov[2] found little variation in the terpene composition of the resin of trees from different geographic sources. All were reported to contain 95–100% β-phellandrene and 0–5% α-pinene; no other terpenes were found. Mirov states that Schorger found only β-phellandrene in earlier analyses. These analyses were made on open-face collected resin using steam distillation and conventional chemical procedures. Williams and Bannister[3] made a gas chromatographic analysis of the terpenes from one *P. contorta* grown in New Zealand; the original source of the tree was not indicated. The resin was collected by the closed-face method and subjected to steam distillation. Their analysis showed a terpene content of 90% β-phellandrene, 2% α-pinene, 4% $\Delta_3$ carene, 3% β-pinene plus myrcene, and a trace of camphene.

In the summer of 1961 resin was collected from one lodgepole pine with a closed-face microtap[4] for studies on the resistance of pines to bark beetles. A few cubic centimetres of the resin were processed with a Hickman still and the distillate was analysed by gas chromatography in the spring of 1962. The chromatogram indicated, as expected, that the major terpene constituent was β-phellandrene. However, it was not present in as large a percentage as previously reported and other terpenes which had not been reported were found. The present paper reports the results of further sampling and qualitative and quantitative analyses made in 1962 using two different chromatographic column supports (LAC[5] and oxydipropionitrile).

## RESULTS

The average values for the terpene composition of nine trees (Table 1) show that β-phellandrene comprised 69·4% of the terpene content of lodgepole pine resin; α-pinene,

[1] W. B. CRITCHFIELD, *Maria Moors Cabot Foundation*, Pub. 3, Harvard Univ. (1957).
[2] N. T. MIROV, *U.S. Dept. Agr. Tech. Bul.* No. 1239 (1960).
[3] A. L. WILLIAMS and M. H. BANNISTER, *J. Pharm. Sci.* 51, 970 (1962).
[4] R. H. SMITH, *J. Econ. Entomol.* 54 (2), 359 (1961).
[5] R. A. BERNHARD and B. SCRUBIS, *J. Chromatog.* 5, 137 (1961).

6·4%; $\Delta_3$ carene, 8·9%; $\beta$-pinene, 5·7%; myrcene, 3·9%; and camphene, 0·5%. Three other terpenes not previously reported in lodgepole pine were found: limonene, 2·4%; sabinene, 2·1%; and $\alpha$-phellandrene, 0·7%. There was also a trace of heptane and an unidentified compound which chromatographed near $\alpha$-pinene with the system used. The nine trees showed generally similar patterns of terpene composition, though there was considerable variation in the percentage of $\beta$-pinene, $\Delta_3$ carene, and $\beta$-phellandrene—in individual trees, especially the first two compounds which in some cases comprised as much as 14–15%.

TABLE 1. TERPENE COMPOSITION OF LODGEPOLE PINE RESIN

| Tree number* | Number of analyses | Heptane | $\alpha$-Pinene | Camphene | $\beta$-Pinene | $\Delta_3$ Carene | Sabinene | $\alpha$-Phellandrene | Myrcene | Limonene | $\beta$-Phellandrene |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Per cent† | | | | | | | | |
| 1-S | 3 | tr | 7·4 | 0·6 | 3·0 | 10·0 | 2·3 | 1·2 | 4·0 | 2·3 | 69·3 |
| 3-S | 2 | — | 5·8 | 0·3 | 5·2 | 14·0 | 2·4 | tr | 4·8 | 3·8 | 63·7 |
| 4-S | 3 | tr | 6·3 | 0·5 | 3·8 | 4·8 | 1·8 | 0·7 | 3·8 | 2·2 | 76·1 |
| 1-T | 6 | tr | 5·0 | 0·5 | 2·8 | 13·7 | 2·5 | 0·9 | 4·1 | 2·4 | 68·1 |
| 2-T | 4 | tr | 6·9 | 0·5 | 9·3 | 6·0 | 2·0 | 0·4 | 4·1 | 2·0 | 68·8 |
| 3-T | 4 | — | 6·5 | 0·4 | 3·3 | 8·0 | 2·2 | tr | 4·0 | 2·6 | 73·0 |
| 4-T | 6 | tr | 6·1 | 0·6 | 7·2 | 9·9 | 2·1 | 1·0 | 3·3 | 2·0 | 67·7 |
| 5-T | 3 | tr | 6·7 | 0·6 | 15·1 | 7·3 | 2·0 | 0·8 | 3·9 | 2·1 | 61·5 |
| 6-T | 3 | — | 6·8 | 0·6 | 1·2 | 6·5 | 1·7 | 0·9 | 3·2 | 2·0 | 77·1 |
| 1-L | 2 | — | 6·5 | tr | 4·6 | 4·6 | 1·5 | — | 3·1 | — | 79·6‡ |
| Average | § | tr | 6·4 | 0·5 | 5·7 | 8·9 | 2·1 | 0·7 | 3·9 | 2·4 | 69·4 |

* S, T, L are location designations for Strawberry, Lake Tahoe, and Leevining respectively.
† Normalized disc integrator values. tr = trace.
‡ Plus limonene and $\alpha$-phellandrene.
§ Without 1-L.

The LAC column did not adequately separate limonene and $\alpha$-phellandrene from the other terpenes. The degree of separation of these two by the oxydipropionitrile column is shown by the chromatograph (Fig. 1). This was made with an unaltered molecular distillate in which a shoulder was evident on $\Delta_3$ carene; and there was a small peak, tentatively identified as $\alpha$-phellandrene, just before myrcene. When 2–4% sabinene (in savin oil) and $\alpha$-phellandrene were added to the distillate, the shoulder on $\Delta_3$ carene became a distinct peak and the small peak before myrcene increased in size, showing that sabinene and $\alpha$-phellandrene were both present. Similarly, when 0·5% camphene and 4% limonene were added, they accentuated the small peaks due to these two constituents.

It is apparent from the results reported here that analyses of pine resin terpenes should be based on more than one tree. The tree source should be noted so that geographic variation

can be detected. Until further comparisons are made it is difficult to estimate the effect of sample collection, preparation, and method analysis on the results. The nature of the terpenes present suggests that closed-face collection and low-temperature preparation are most suitable.

## EXPERIMENTAL

Resin samples were collected with a closed-face microtap from lodgepole pine (*P. contorta* var. *murrayana* (Grev. and Balf.) Engelm.) in three locations in California: (1) at Strawberry, on U.S. Route 50, (2) near the south shore of Lake Tahoe, and (3) on California Route 120, west of Leevining. Three of the trees from the Lake Tahoe area had been used previously by Mirov.[2] Samples were prepared for analysis by ether or ethanol

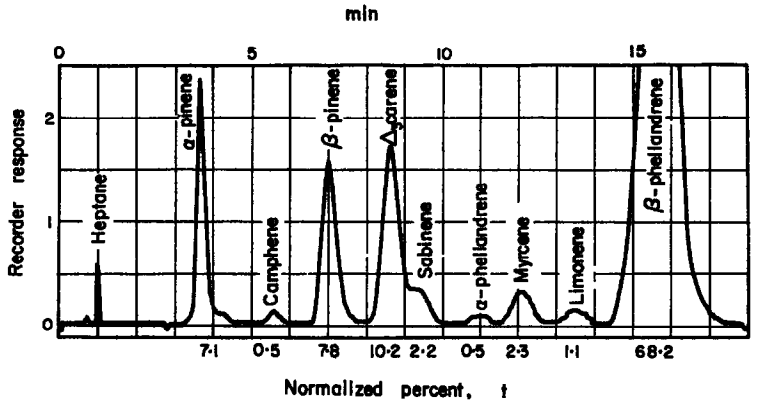


FIG. 1. GAS CHROMATOGRAPHS OF THE MOLECULAR DISTILLATE OF LODGEPOLE PINE RESIN OBTAINED WITH A 10% OXYDIPROPIONITRILE COLUMN.

extraction of fresh resin or by processing the resin with a Hickman molecular still at 40° for 24 hr at atmospheric pressure.

Analyses were made on a sensitive gas chromatograph (Aerograph A-90-P), using two recently tested columns. The operational conditions were: (1) temperatures of 120–130° on the injector, 60° on the column, and 150° on the detector; (2) filament current of 200 mA; (3) helium flow of 90 and 50 ml/min at the outlet port; (4) sample size of 0·4–2·0 $\mu$l; and (5) columns of 8-ft by $\frac{1}{4}$-in. stainless steel: solid support of 60/80 acid-washed Chromosorb W, and liquid supports of 20% LAC-446[5] (adipate polyester of diethylene glycol partially cross-linked with pentaerythritol) and oxydipropionitrile[6] at both 10 and 20%. Each sample was analysed immediately after preparation and at one or more times in the next 6 months.

Qualitative determinations were made by comparing relative retention times for the two columns with literature data and with known compounds, and by internal standardization with the known compounds. Quantitative determinations were made by internal normalization of disc integrator values; checks with synthetic mixtures of known percentage composition proved this to be a valid procedure.

Analyses were made of each resin with the oxydipropionitrile columns. Comparisons with the LAC column showed that the latter failed to separate two of the minor constituents

[6] M. H. KLOUWEN and R. TER HEIDE, *J. Chromatog.* 7, 297 (1962).

adequately, though the quantitative comparisons were similar if values obtained for the individual terpenes with the oxydipropionitrile column were grouped to represent the corresponding unseparated constituents as obtained on the LAC column. No difference could be attributed to type of sample preparation, size of sample, rate of helium flow, or length of holding the sample. The sample from Leevining was extracted with ethanol, and since the oxydipropionitrile column does not differentiate ethanol and $\beta$-pinene, it could be analysed on the LAC column only.