acetaldehyde fraction was accomplished with alkaline potassium permanganate, and the presence of N,N-dimethylglycine in the products was confirmed by paper chromatography. The total crude mixture containing the N,N-dimethylglycine was absorbed onto calcium oxide and pyrolized, and the trimethylamine produced was collected in methanolic hydrogen chloride. A sample of trimethylamine picrate was prepared to confirm the identity of the amine. Specific activity of the amine salt was 0.402 μcurie/mmole (61% of the activity of II). Hence, 61% of the carbon-14 was incorporated into C-5 of the pyrrolidine ring adjacent to the nitrogen atom. Ozonolysis of *p*-methoxycinnamaldehyde (III, 0.285 μcurie/mmole) gave anisaldehyde, hydrolysis of which, with hydrobromic acid, produced methyl bromide, which was distilled from the reaction mixture into methanol. The methyl bromide solution represented 91% of the radioactivity of III. Thus, [2-$^{14}$C]-glycine is incorporated into two parts of the anisomycin molecule. Some of it is metabolized to formic acid and formaldehyde and enters the pool of one-carbon fragments and is subsequently used to methylate the phenolic hydroxyl of the tyrosine, and some glycine becomes incorporated into the pyrrolidine ring. The location of the labeled carbon atom from glycine at C-5 of the pyrrolidine ring suggests that the adjacent carbon atom at C-4 is derived from the glycine carboxyl group.

[Me-$^{14}$C]L-Methionine gave rise to deacetylanisomycin (II) with a specific activity of 7.6 μcuries/mmole. Degradation of II gave *p*-methoxycinnamaldehyde (III) containing 7.8 μcuries/mmole; hence, the dimethylaminoacetaldehyde (IV) could not be radioactive. Hydrolysis of the dinitrophenylhydrazone of III proceeded in a most unsatisfactory manner. The derivative was not very soluble in hydrobromic acid, and there was some loss of material through bumping despite the stream of nitrogen which was bubbled through the reaction mixture. The methyl bromide which was collected represented only 33% of the radioactivity of III. This low yield of radioactivity is probably due to bumping and low boiling point of methyl bromide (4.5°), since it is not plausible that 70% of the labeled methionine was incorporated into the unsaturated side chain of III. Indeed, we have already shown that two of these carbon atoms were derived from phenylalanine or tyrosine, and the third carbon atom has a higher oxidation state than would be expected for a group derived from the labeled methyl group of methionine. Methionine probably plays its normal role as a biological methylating agent in the biosynthesis of anisomycin and gives rise to the methoxy group of the aromatic ring.

Fermentations in the presence of labeled acetate usually produced anisomycin with a low specific activity. This was due to the dilution of isotope by the large amounts of acetic acid produced in the course of the fermentation. Sodium [1-$^{14}$C]acetate provided anisomycin (I) with a specific activity of 0.511 μcurie/mmole. Hydrolysis of this sample gave deacetylanisomycin (II) with 0.285 μcurie/mmole; thus, 44% of the radioactivity was present in the acetoxy group. Therefore, acetic acid, probably as acetyl coenzyme A, is used for the acetylation of deacetylanisomycin (II) to produce anisomycin (I).

Degradation of the labeled deacetylanisomycin from [1-$^{14}$C]acetate was not continued further. However, II from [2-$^{14}$C]acetate was degraded to *p*-methoxycinnamaldehyde which had no significant amount of radioactivity. Acetate appears to be incorporated into the pyrrolidine ring at C-4 and C-5. Further studies with labeled acetate were not possible because of the poor incorporation into the anisomycin owing to isotope dilution and the low yields of the antibiotic.

Thus, we have been able to demonstrate that tyrosine, glycine, methionine, and acetate are precursors for the biosynthesis of anisomycin. Phenylalanine is utilized to a limited degree. Tyrosine donates C-2 of the pyrrolidine ring, and C-4 and C-5 are derived from acetate or glycine. The origin of C-3 was not determined.

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## Presence of Sandaracopimaric and $\Delta^{8(9)}$-Isopimaric Acids in Pine Oleoresin

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Sandaracopimaric and $\Delta^{8(9)}$-isopimaric acids[2] were isolated from pine oleoresin.

Sandaracopimaric acid has previously been detected in a number of sources.[3] von Rudloff and Sato have detected it in trace amounts from the heartwood extracts of *Pinus banksiana*[4] and also in *Pinus resinosa* Ait.[5] The $\Delta^{8(9)}$-isopimaric acid has been detected in small amounts in the resins from *Tetraclinis* and *Callitris* species and in trace amounts in samples of isopimaric acid isolated from *Dacrydium biforme* Pilger. Genge[6] suggested that one of the peaks in their mass spectra indicated the presence of $\Delta^{8(9)}$-isopimaric acid in some rosins. This is the first report, however, of either acid as a constituent of pine oleoresin.

In our work on gas chromatographic analysis of pine gums and rosins several unidentified peaks were observed. The components of two of these peaks were collected in large enough quantities for identification. The first peak off the gas chromatograph (see Table I) was identified as $\Delta^{8(9)}$-isopimaric acid. The fourth peak off the column was identified as sandaracopimaric acid.

Since the $\Delta^{8(9)}$-isopimaric acid was known to be an acid isomerization product of isopimaric acid,[7] it was suspected that the acid used to stimulate the flow of gum from the tree might be causing this isomeriza-

(1) One of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.
(2) Steroid numbering.
(3) L. J. Gough, *Chem. Ind.* (London), 2051 (1964).
(4) E. von Rudloff and A. Sato, *Can. J. Chem.*, **41**, 2165 (1963).
(5) A. Sato and E. von Rudloff, *ibid.*, **42**, 635 (1964).
(6) C. A. Genge, *Anal. Chem.*, **31**, 1750 (1959).
(7) O. E. Edwards and R. Howe, *Can. J. Chem.*, **37**, 760 (1959).

TABLE I

COMPOSITION OF RESIN ACID FRACTION OF A TYPICAL SAMPLE OF SLASH PINE OLEORESIN
(*Pinus elliottii* VAR. *elliottii*)

| Peak no. | Methyl ester of | % of oleoresin[a] | % of total acids[b] | r[c] | α[d] |
|---|---|---|---|---|---|
| | Arachidic acid[e] | | | 1.00 | |
| 1 | $\Delta^{8(9)}$-Isopimaric acid | <1 | 1 | 1.30 | |
| 2 | Elliotinoic acid | 2.7 | 3.8 | 1.39 | 1.07 |
| 3 | Pimaric acid | 3.2 | 4.5 | 1.43 | 1.03 |
| 4 | Sandaracopimaric acid | 1.1 | 1.5 | 1.59 | 1.11 |
| 5 | Levopimaric and palustric acids | 26.9 | 37.5 | 1.75 | 1.10 |
| 6 | Isopimaric acid | 14.9 | 20.7 | 1.90 | 1.09 |
| 7 | Unidentified acid | 1 | 1.4 | 2.07 | 1.09 |
| 8 | Dehydroabietic acid | 2.5 | 3.5 | 2.21 | 1.07 |
| 9 | Abietic acid | 6.9 | 9.6 | 2.53 | 1.14 |
| 10 | Neoabietic acid | 13.0 | 18.1 | 2.92 | 1.15 |

[a] Per cent of oleoresin based on weight of the whole oleoresin sample. [b] Per cent of total acid based on the acids off the column. This value agreed within 5% of the value obtained by titration. [c] The retention value for methyl arachidate was taken as 1.00 and the values for the other esters are given relative to that of methyl arachidate. [d] The separation factor ($\alpha$) is the ratio between the retention value of one peak and that of the preceding peak. [e] The methyl ester of arachidic acid was used as an internal standard and also to determine the relative retention values. The relative response of each ester was determined by dividing the area of the methyl arachidate peak into the area of the ester peak as described by Brooks, *et al.*[9]

tion. However, the $\Delta^{8(9)}$-isopimaric acid was present in the same quantities in the oleoresin from freshly streaked trees on which no acid had been used. In order to show that the two new acids were not artifacts formed on the g.l.p.c. column, pure samples of methyl isopimarate and methyl pimarate were put through the gas chromatograph, collected, and rechromatographed. Single peaks with retention values identical with those of the starting esters were observed.

In most rosins the $\Delta^{8(9)}$-isopimaric acid accounted for less than 1% of the acid fraction. A rosin sample from the pinyon pine (*Pinus edulis* Engelm), however, contained this acid as the major acid component (more than 30%). Sandaracopimaric acid was present in all rosins examined and accounted for about 1–2% of the acid fraction. The probably inevitable occurance together of isopimaric acid, sandaracopimaric acid, and $\Delta^{8(9)}$-isopimaric acid indicates that they are formed from a common C-8 carbonium ion precursor with the relative amounts of each product varying widely from one source to another. The three analogous pimaric acid isomers would similarly be expected but as yet have not been isolated.

Sandaracopimaric acid has been found to be a contaminant of most samples of pimaric acid. Pure pimaric acid has a melting point of 217–219°.[8] A 5:1 mixture of pimaric–sandaracopimaric acids melts at 212–213°. The mixture is not readily separated by recrystallization. The phase diagram (Figure 1) shows the absence of a melting point depression in mixtures of these acids.

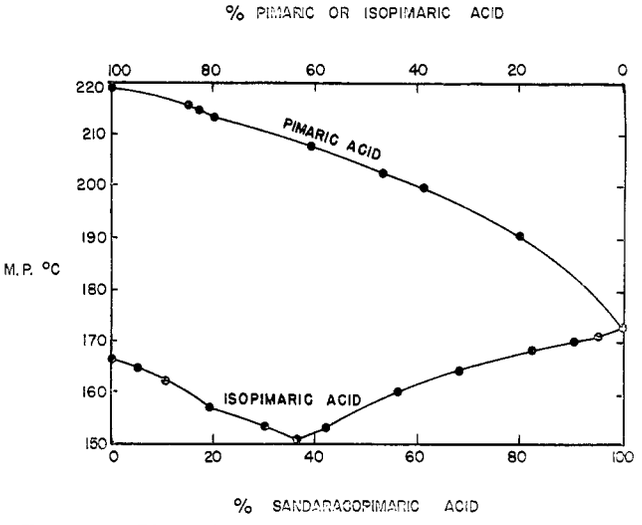


Figure 1.—Phase diagram of melting points of mixtures of pimaric *vs.* sandaracopimaric acids and isopimaric *vs.* sandaracopimaric acids.

### Experimental Section

An F & M gas chromatograph, Model 700, equipped with a thermal-conductivity detector, was used for this investigation. A 15 ft. × 0.25 in. copper tube packed with 5% Versamid 900 on Chromosorb W (60–80 mesh) was used for the chromatographic column.[9] The following operating conditions were used: column temperature, 245°; injection chamber and detector block temperature, 300°; and helium flow, 150 cc./min.

The resin acids were converted to their methyl esters for gas chromatographic analysis. The unidentified peaks were collected in 2-mm. (i.d.) glass collection tubes and were transferred directly to microplates for infrared spectra. The collection tubes were emptied and the oils were crystallized from methanol. The crystalline esters had the following physical constants: **methyl $\Delta^{8(9)}$-isopimarate**, $[\alpha]_D$ +118° (*c* 1.0, alcohol), m.p. 68–70° (mixture melting point with authentic sample showed no depression), infrared spectrum 1724 ($COOCH_3$), 3025, 1635, and 905 cm.$^{-1}$ (vinyl); **methyl sandaracopimarate**, $[\alpha]_D$ −20.2° (*c* 1.0, alcohol), m.p. 66–68° (mixture melting point with authentic sample showed no depression), infrared spectrum 1722 ($COOCH_3$), 3030, 1625, and 908 cm.$^{-1}$ (vinyl).

Sandaracopimaric acid was found in all pine oleoresins and rosins examined and varied from 0.7 to 2.5% of the gross weight of the sample. The average content in ten samples of slash oleoresin was 1.2%. The $\Delta^{8(9)}$-isopimaric acid was found in all samples of slash and longleaf pine oleoresin and rosin examined. The average content for slash pine oleoresin was about 0.5%.

(8) G. C. Harris and T. H. Sanderson, *J. Am. Chem. Soc.*, **70**, 2081 (1948).

(9) T. W. Brooks, G. S. Fisher, and N. M. Joye, *Anal. Chem.*, **37**, 1063 (1965).