

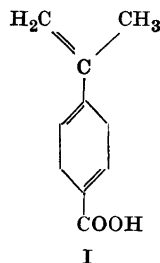
## Antibiotic Substances from the Heart Wood of *Thuja plicata* D. Don

### VI.\* The Structure of Thujic Acid (Dehydroperillic Acid)

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In 1933 Anderson and Sherrard<sup>1</sup> attributed formula I to an acid occurring in the heart-wood of western red cedar (*Thuja plicata* D. Don.).



As it contains one double bond more than perillic acid, they termed the acid dehydroperillic acid. Their chief arguments in favour of this formula were the conversion of the acid into cumic acid by boiling with 3 % hydrochloric acid; and the results of ozonisation experiments which yielded, among other compounds, a substance believed to be  $\beta$ ,  $\gamma$ -diketovaleric acid.

In this connection it might be mentioned that the author has been unable to duplicate this conversion with 3 % hydrochloric acid as described by Anderson and Sherrard. Boiling with conc. hydrochloric acid in acetic acid solution, however, gave cumic acid in good yield.

\* Part V. Preliminary note. Erdtman, H., and Gripenberg, J. *Nature* **164** (1949) 316.

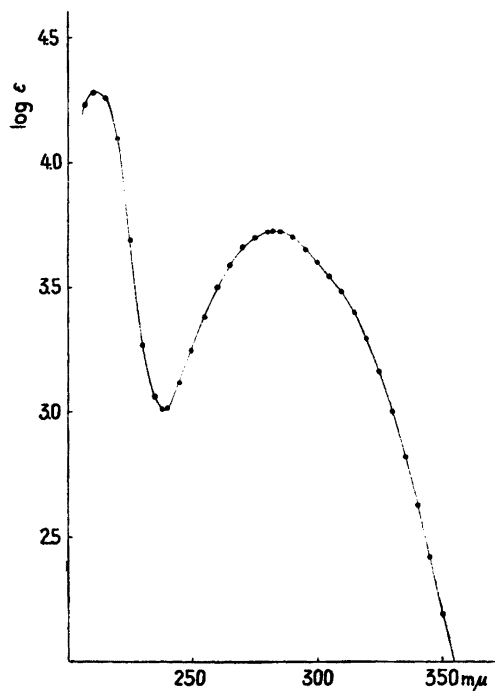


Fig. 1. Absorption spectrum of thujic acid.

In a preliminary communication <sup>2</sup>, Erdtman and the present author pointed out that the absorption spectrum of the acid from *Thuja plicata* (Fig. 1) is not compatible with structure I, but indicates a larger conjugated system. Quite recently Hurd and Edwards <sup>3</sup> have also expressed some doubts as to the validity of this structure. Also in agreement with their observations is the failure of 'dehydroperillic acid' to react with maleic anhydride.

A closer investigation showed that the acid possesses a quite different carbon skeleton. The formation of cumic acid is due to a molecular rearrangement. It has therefore been proposed <sup>4</sup> that the name 'dehydroperillic acid' should be abandoned, as there is no structural relationship to perillic acid, and the acid renamed *thujic acid*.

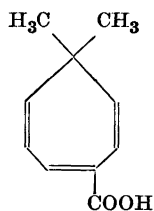
Anderson and Sherrard <sup>1</sup> prepared hexahydrothujic acid by catalytic hydrogenation of thujic acid. The oily product was believed to be a previously unknown liquid isomeride of hexahydrocumic acid of m.p. 94—95°. Cooke and Macbeth <sup>5</sup>, however, later prepared this isomeride and characterised it by conversion into the *p*-bromophenacyl ester m.p. 85°. Hexahydrothujic acid, however, yielded a *p*-bromophenacyl ester which was obtained in two dimorphic modifications, one with m.p. 70—71°, the other with m.p. 65—66°.

A mixed melting point determination with the *p*-bromophenacyl ester of hexahydrocuminic acid prepared according to Cooke and Macbeth<sup>5</sup> showed strong depression.

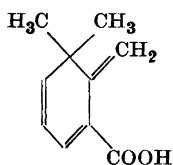
Thus hexahydrothujic acid is not identical with either of the two possible forms of hexahydrocuminic acid, and hence, cannot have the carbon skeleton of I.

Furthermore, on oxidation with potassium permanganate, thujic acid gave dimethylmalonic acid. This indicates the presence of a *gem*-dimethyl group in the molecule of thujic acid.

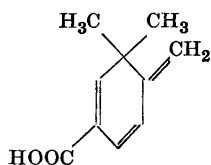
Taking into account the formation of dimethylmalonic acid on oxidation, and the facile isomerisation of thujic acid to cumic acid, the following three formulae suggest themselves as possible structures of thujic acid.



II



III



IV

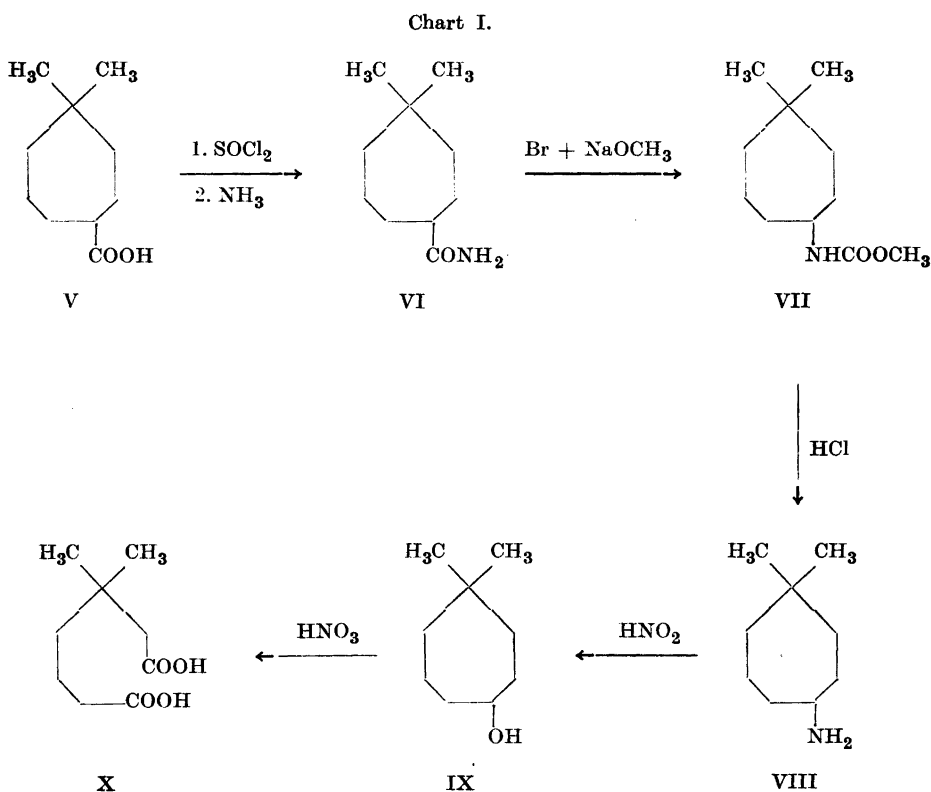
Anderson and Sherrard<sup>1</sup> claim the isolation of formaldehyde as a product of ozonisation of thujic acid, but do not mention the yield. This would, tend to indicate either III or IV as the structure of thujic acid. The author, however, has only been able to detect traces of formaldehyde on ozonisation and it is well known that formaldehyde may be obtained in small amounts from many compounds which do not contain methylene groups. The formation of only one hexahydrothujic acid is in harmony with formula II. III and IV would certainly give rise to two diastereoisomerides. Furthermore, C-CH<sub>3</sub>-estimations on both thujic acid and hexahydrothujic acid gave only about 0.5 C-CH<sub>3</sub> groups. Hexahydroderivatives of III or IV would be expected to give a value well above 1 C-CH<sub>3</sub>-group. Thus, there are strong indications in favour of structure II for thujic acid.

The degradation reactions described below clearly show that this is the correct structure of thujic acid, which consequently is 4,4-dimethylcyclohepta-2, 5, 7-trienecarboxylic acid.

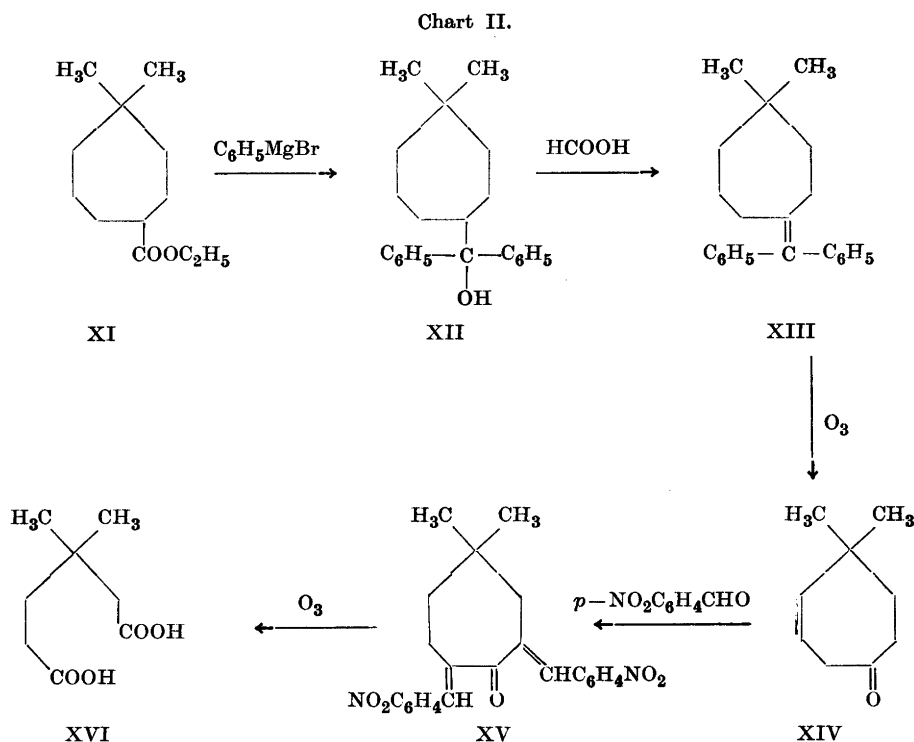
The degradation of thujic acid was first attempted through the tetrahydrothujic acid, which can be obtained from thujic acid by reduction with sodium amalgam in alkaline solution. The yield of tetrahydrothujic acid was,

however, inconsistent. The tetrahydrothujic acid was often accompanied by sparingly soluble, probably high molecular products. Furthermore, preliminary experiments on the degradation of tetrahydrothujic acid were not very encouraging, and this route was therefore abandoned. The position of the remaining double bond in tetrahydrothujic acid remains undetermined.

Hexahydrothujic acid proved to be more suitable for degradation studies and the degradation was carried out along two different lines, both leading to previously known compounds. The steps are outlined in Chart I and II.



The last step should give a mixture of  $\beta$ ,  $\beta$ -dimethylpimelic acid and  $\gamma$ ,  $\gamma$ -dimethylpimelic acid. Only one crystalline substance, however, could be isolated, the mother-liquor being a noncrystallisable oil. The former was identified as  $\beta$ ,  $\beta$ -dimethylpimelic acid by means of mixed melting point determination with an authentic sample prepared according to Blanc<sup>6</sup>.



The last step gave only a rather small amount of an acid, which could not be obtained quite pure. It was, however, identified as  $\beta, \beta$ -dimethyladipic acid by mixed melting point determination with an authentic sample prepared by oxidation of 4,4-dimethylcyclohexanone.

The isolation of  $\beta, \beta$ -dimethylpimelic acid and  $\beta, \beta$ -dimethyladipic acid definitely establishes the structure of hexahydrothujic acid as V and, as there is only one way of arranging three double bonds in this molecule, thujic acid must have structure II.

Hence, it is a higher homologue of cyclohepta-1,3,6-trienecarboxylic acid. Four isomeric cycloheptatrienecarboxylic acids are known and Buchner<sup>7</sup> has attributed this structure to the liquid isomeride prepared by Einhorn and Willstätter<sup>8</sup>.

The formula V explains all the reactions of thujic acid, and also the isomerisation to cumic acid. This resembles the transformation of eucarvone into carvacrol<sup>9</sup> and cycloheptatriene into benzylbromide.<sup>10</sup>

It is interesting to note that both the thujaplicins and thujic acid, all occurring in the heart wood of *Thuja plicata*<sup>11</sup>, contain seven-membered rings.

Of course there is a great difference between the tropolone ring system of the thujaplicins and the seven-membered ring in thujic acid, but they are probably all biochemically related substances. Thujic acid contrary to the thujaplicins, does not show any 'aromatic' properties. As a matter of fact, thujic acid is rather unstable and decomposes on standing. Anderson and Sherrard noted that thujic acid is slightly toxic to wood-destroying fungi, and this is in agreement with recent findings of E. Rennerfelt, Stockholm.

### EXPERIMENTAL

#### Isolation of thujic acid

The powdered heart wood was extracted with acetone and the extract segregated into acidic, phenolic and neutral fractions as described earlier<sup>11</sup>.

The sodium hydrogen carbonate solution was acidified and the precipitated crude thujic acid was collected with ether. After removal of the ether, the crude thujic acid was distilled with steam. The crystalline precipitate in the receiver was filtered, dried and recrystallised from light petroleum. M. p. 88–89°.

$C_{10}H_{12}O_2$	Calc.	1 C-CH <sub>3</sub>	9.15
	Found	C-CH <sub>3</sub>	6.91

#### Isomerisation of thujic acid

Thujic acid (0.5 g) was suspended in 10 ml conc. hydrochloric acid, and the solution heated to boiling. Acetic acid was added until a clear solution was obtained. The boiling was continued for half an hour, and the solution then poured into water. The white precipitate was filtered and recrystallised from dilute methanol (m. p. 115–116°). No depression when mixed m. p. was taken with an authentic sample of cumic acid.

#### Oxidation of thujic acid

Thujic acid (1 g) was dissolved in dilute sodium carbonate solution and potassium permanganate was added until the colour remained. The reaction mixture was then acidified and sulphur dioxide passed in, in order to dissolve the manganese dioxide. The clear colourless solution was extracted with ether. The ether solution yielded 0.2 g of a crystalline substance, which after recrystallisation from ether and sublimation in a vacuum showed a m. p. of 190–192°.

$C_3H_6(COOH)_2$	Calc.	Eqv. wt.	66.0
	Found	»	»

Upon heating, carbon dioxide was evolved. The remaining oily acid was converted into the *p*-bromophenacyl ester, m. p. 75–76°, undepressed on admixture of an authentic sample of isobutyric acid *p*-bromophenacyl ester.

#### Tetrahydrothujic acid

Thujic acid (4 g) was dissolved in sodium hydrogen carbonate solution. The solution was heated on a waterbath to about 70–80° and 4 % sodium amalgam (75 g) was in-

roduced in small portions. The heating was continued, with frequent shaking, for eight hours. The solution was decanted from the mercury, acidified, and extracted with ether. After evaporation of the latter, 4 g of an oil was obtained, which soon partly crystallised. The crystals were filtered and recrystallised from dilute methanol. The tetrahydrothujic acid thus obtained had m. p. 65–66°. After standing for some months the crystals were converted into a thick colourless oil.

$C_{10}H_{16}O_2$	Calc.	C	71.34	H	9.61
	Found	»	71.49	»	9.61 (W. K.)

The *p*-bromophenacyl ester was prepared in the usual way and after recrystallisation from dilute methanol had a m. p. of 74–75°.

$C_{18}H_{21}O_3Br$	Calc.	C	59.16	H	5.81
	Found	»	58.93	»	5.74 (W. K.)

The benzylisothiuronium salt was prepared in the usual way and after recrystallisation from alcohol had a m. p. of 167–168°.

$C_{18}H_{26}O_2N_2S$	Calc.	C	64.61	H	7.86
	Found	»	64.04	»	7.90 (W. K.)

#### Hydrogenation of tetrahydrothujic acid

Tetrahydrothujic acid (0.3 g) was hydrogenated in alcohol solution over a platinum oxide catalyst. The uptake of hydrogen amounted to 40 ml (calculated for 1 mol 40 ml). The hydrogenated acid was obtained as an oil, which was converted into the *p*-bromophenacyl ester, m. p. 70–71°. This gave no depression with the *p*-bromophenacyl ester of hexahydrothujic acid described below.

#### Hexahydrothujic acid (V)

Thujic acid (1 g) was hydrogenated in alcohol over platinum oxide catalyst. 395 ml of hydrogen (calculated for 3 moles 410 ml) were absorbed. The alcohol was removed in a vacuum and the oily product distilled. B. p. 150–152°/14 mm,  $n_D^{20}$  1.4671. It could not be made to crystallise.

$C_{10}H_{18}O_2$	Calc.	C	70.50	H	10.68	C-CH <sub>3</sub>	8.82
	Found	»	70.19	»	10.53	»	4.03 (W. K.)

The *p*-bromophenacyl ester was obtained in two dimorphic modifications, one long needles (m. p. 70–71°), and the other leaflets (m. p. 65–66°).

$C_{18}H_{23}O_3Br$	Calc.	C	58.83	H	6.33
	Found	»	59.07	»	6.34 (W. K.)

The benzylisothiuronium salt had m. p. 159–160° after recrystallisation from alcohol-ethyl acetate.

$C_{18}H_{28}O_2N_2S$	Calc.	C	64.23	H	8.41
	Found	»	64.33	»	8.38 (W. K.)

The amide (VI) was obtained by heating the hexahydrothujic acid with thionyl chloride, removing the excess thionyl chloride by distillation, and pouring the residue into conc. ammonia. Recrystallisation from dilute alcohol yielded leaflets m. p. 147.5–148.5°.

The ethyl ester (XI) was obtained by boiling the alcoholic solution obtained in the hydrogenation with a few drops of conc. sulphuric acid. B. p. 107–107.5°/11 mm.

#### 4,4-Dimethylcycloheptylamine (VIII)

Hexahydrothujic amide (2.7 g) was added to a solution of sodium (0.75 g) in 40 ml methanol. Bromine (2.52 g) was then added dropwise. The mixture was warmed for a few minutes on a water bath, made slightly acidic with acetic acid, and the methanol evaporated. Water was added and the urethane (VII) extracted with ether. It was obtained as an oil which was, without further purification, hydrolysed with conc. hydrochloric acid on a water bath. A small amount of a nonbasic material was removed by extraction with ether and the hydrochloric acid solution was evaporated to dryness, when the hydrochloride of 4,4-dimethylcycloheptylamine (2.2 g) was obtained. The free base was an oil which was characterised as its benzoate, colourless crystals from ligroin m. p. 114–115°.

$C_{16}H_{23}ON$	Calc.	C	78.51	H	9.47
	Found	»	78.83	»	9.52 (W. K.)

#### $\beta,\beta$ -Dimethylpimelic acid (X)

4,4-Dimethylcycloheptylamine hydrochloride (1.1 g) was dissolved in a small amount of water and sodium nitrite (0.36 g) was added. The solution was heated on a water bath until the evolution of gas ceased. The solution separated into two layers. The alcohol (IX) formed was collected with ether. On removal of the ether, 0.45 g of the alcohol was obtained as an oil. This was oxidised, without further purification by heating with conc. nitric acid. The nitric acid was evaporated in a vacuum. There remained a thick colourless oil, which partly crystallised. The crystals were collected by filtration (0.1 g) and recrystallised from light petroleum — ether. M. p. 101–102°. Mixed m. p. with authentic  $\beta,\beta$ -dimethylpimelic acid<sup>6</sup> of m. p. 102–104° was 102–103°.

$C_9H_{16}O_4$	Calc.	C	57.38	H	8.59
	Found	»	57.48	»	8.68 (K. S.)

#### 1-Diphenylmethylene-4,4-dimethylcycloheptane (XIII)

Ethyl hexahydrothujate (5 g) was added dropwise to a Grignard-reagent prepared from bromobenzene (12 g) and magnesium (1.75 g) in anhydrous ether. After the addition of the ester, the mixture was boiled for two hours and then allowed to stand over night. It was then poured on ice and acidified with sulphuric acid. The reaction product was extracted with ether, the ether removed, and the remaining oil (probably mostly XII)



boiled for two hours with 85 % formic acid. The mixture was then poured into water and extracted with ether. The ether extract was washed with sodium hydrogen carbonate solution. Evaporation of the ether left a thick yellow oil, which soon partly crystallised. The crystals were filtered and recrystallised from alcohol. Yield 5.1 g, m. p. 89—90°.

$C_{22}H_{26}$	Calc.	C	90.94	H	9.06
	Found	»	90.96	»	8.80 (K. S.)

#### 4,4-Dimethylcycloheptanone (XIV)

1-Diphenylmethylene-4,4-dimethylcycloheptane (4 g) was ozonised in chloroform solution. The chloroform was evaporated in a vacuum and the ozonide decomposed by boiling with water. The 4,4-dimethylcycloheptanone was separated by steam distillation from benzophenone, which is only slightly volatile with steam. Extraction of the distillate with ether yielded 0.6 g of the ketone, B. p. 60—66°/10 mm. The 4,4-dimethylcycloheptanone was characterised as its semicarbazone. This was first obtained as long needles m. p. 191—192°, but on further recrystallisation from methanol leaflets were obtained m. p. 173—174°. Thereafter only the lower melting form could be obtained.

$C_{10}H_{19}ON_3$	Calc.	C	60.85	H	9.73
	Found	»	60.94	»	9.66 (K. S.)

#### Bis-*p*-nitrobenzylidene-4,4-dimethylcycloheptanone (XV)

4,4-Dimethylcycloheptanone (0.3 g) was dissolved in alcohol and *p*-nitrobenzaldehyde (0.7 g) was added. The mixture was warmed slightly in order to dissolve all of the aldehyde. A few drops of a sodium methoxide solution were added. After four days, the crystals deposited were collected (0.7 g) and recrystallised from alcohol-ethyl acetate. This resulted in small yellow needles m. p. 158—160°.

$C_{23}H_{22}O_5N_2$	Calc.	C	67.95	H	5.47
	Found	»	67.51	»	5.63 (K. S.)

#### $\beta,\beta$ -Dimethyladipic acid (XVI)

The bis-*p*-nitrobenzylidene-4,4-dimethylcycloheptanone (0.5 g) was ozonised in chloroform solution until the yellow colour had disappeared. The chloroform was evaporated in a vacuum and the remaining oil boiled with water. Sodium hydrogen carbonate was added and the insoluble *p*-nitrobenzaldehyde was removed by filtration. The aqueous solution was acidified and a small amount of *p*-nitrobenzoic acid removed. The mother liquor was extracted with ether, the ether evaporated, and the residue treated with water. An additional amount of *p*-nitrobenzoic acid could thus be removed. The aqueous solution was evaporated to dryness, leaving an oil which soon crystallised. This was recrystallised from a mixture of ether and light petroleum and small needles m. p. 82—83° were obtained. The yield was 0.02 g. Mixed with authentic  $\beta,\beta$ -dimethyladipic acid of m. p. 85—86° it melted 84—85°.

## SUMMARY

The 'dehydroperillic acid' of Anderson and Sherrard<sup>1</sup> has been shown to be 4,4-dimethylcyclohepta-2,5,7-trienecarboxylic acid, and the name thujic acid is proposed for this acid.

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The analyses were carried out by W. Kirsten, Upsala, (W. K.) and K. Salo, Helsingfors, (K. S.).

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