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

IV.* The Constitution of β-Thujaplicin

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In a joint communication with Sherrard[1] one of the present authors (A. B. A.) described the isolation of »dehydroperillic acid» and a »phenol», m. p. 82°, $C_{10}H_{12}O_2$, now termed γ-thujaplicin[2], from the heart wood of western red cedar (*Thuja plicata* D. Don). The crystalline »phenol», however, constituted only part of the »phenolic» fraction of the volatile oil obtained by steam distillation of the wood. The remainder was an oil possessing approximately the same composition as γ-thujaplicin. In the course of time (16 years have elapsed) the oil had partly crystallised. Purification of the crystals readily yielded a pure compound m. p. 52—52.5° having the composition $C_{10}H_{12}O_2$. Hence, it is an isomeride of »dehydroperillic acid», α-and γ-thujaplicin and has been termed β-thujaplicin for reasons given below. It exhibits the same green ferric colour reaction as α- and γ-thujaplicin and gives a chloroform soluble copper complex. Its U. V. absorption curve (Fig. 1, Part II) is also very similar to those of α- and γ-thujaplicin. Consequently a close structural relationship between β-thujaplicin and the two other thujaplicins was expected.

The degradation of β-thujaplicin, therefore, followed the route outlined in Part II. β-Thujaplicin, like γ-thujaplicin, on catalytic hydrogenation consumed 4 moles of hydrogen, yielding a thick oil, which could not be obtained

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crystalline. This oil may be a mixture of diastereoisomeric octahydro-β-thujaplicins. The composition of this product corresponded to $C_{10}H_{20}O_2$ (II). It reduced periodic acid and on oxidation with potassium permanganate it yielded a dicarboxylic acid $C_{10}H_{18}O_4$ (III). This also was obtained only as an oil, but was characterised as the bis-benzylthiuronium salt, m. p. 152.5—153°. Dry distillation of the barium salt of the acid yielded an oil showing ketonic properties and a smell resembling that of menthone. From this a 2,4-dinitrophenylhydrazone, m. p. 139—140° and a semicarbazone, m. p. 187—188° were obtained. These figures agree fairly well with those given by Whitmore and Pedlow [3] (139—140° and 189—190° resp.) for the corresponding derivatives of 3-*iso*propyl*cyclo*hexanone (IV).

β-Thujaplicin like α- and γ-thujaplicin gives *iso*butyric acid on careful oxidation with chromic acid. Therefore it could be anticipated that the acid $C_{10}H_{18}O_4$ should be the hitherto unknown β-*iso*propylpimelic acid. This was synthesised by the following route:


III
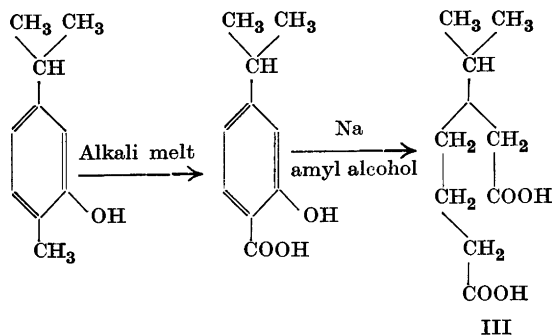
The oily dicarboxylic acid obtained, was characterised as its bis-benzyl-thiuronium salt m. p. 152.5—153°, undepressed when admixed with the corresponding salt of the acid obtained by degradation of β-thujaplicin.

It follows that β-thujaplicin possesses structure I and the degradation reactions are outlined in the scheme on page 566.

The name β-thujaplicin refers to the position of the *iso*propyl group relative to the oxygen atoms.

Regarding the physico-chemical aspects of the formulation of β-thujaplicin compare Part II [2].

Like α- and γ-thujaplicin, β-thujaplicin is highly toxic towards wood destroying fungi. A full account of the mycological aspect of these substances will be published elsewhere by E. Rennerfelt. As a matter of fact β-thujaplicin appears to be slightly more toxic than α- and γ-thujaplicin.
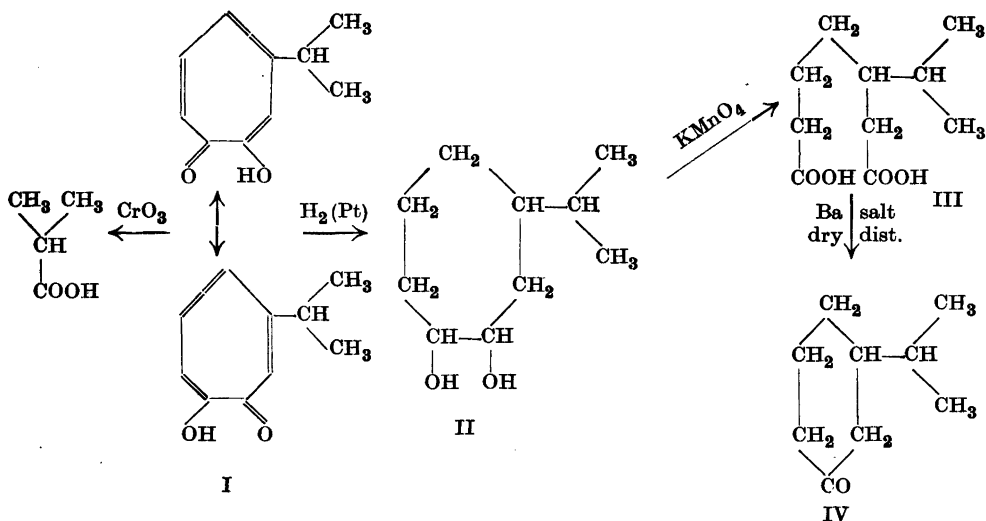
As already pointed out in Part II the yield of the thujaplicins varies considerably in different samples of heart wood. A study of the distribution of the thujaplicins (β- and γ-thujaplicin) in two different trees was carried out by one of us (A. B. A. 1932) in the Forest Products Laboratory, Madison, Wisconsin, in connexion with the early investigation by Anderson and Sherrard [1]. Sections from a tree were obtained from Portland, Oregon, which had been in a mill pond for some time and the other tree was obtained from Snoqualmie Falls, Washington. The latter tree was immediately sent to the laboratory after being felled.

The presence of the thujaplicins was determined by testing the toxicity to *Fomes annosus* of the steam distillate of the samples obtained as described in the experimental part.

The results are given in the following tables:

Table 1. Distribution of toxic principle in tree from Snoqualmie Falls, Washington.

Distance from center of butt of log	Colour with alcoholic $FeCl_3$	Killing or inhibition point (10 ml = 1 g wood)	Section 15 feet from butt of log (distance from center)	Colour with alcoholic $FeCl_3$	Killing or inhibition point (10 ml = 1 g wood)
Inches		ml	Inches		ml
0—4	Intense green ...	10	0—4	Intense green ..	10
4—8	...do ...	12	4—8	...do ..	10
8—12	...do ...	12	8—12	...do ..	10
12—16	...do ...	10	12—16	...do ..	10
16—20	...do ...	10	16—20	...do ..	10

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Table 2. *Distribution of toxic principle in tree from Portland, Oregon.*

Distance from center of butt of log	Colour with alcoholic $FeCl_3$	Killing or in-hibition point (10 ml = 1 g wood)	Section 15 feet from butt of log (distance from center)	Colour with alcoholic $FeCl_3$	Killing or in-hibition point (10 ml = 1 g wood
Inches		ml	Inches		ml
0—3	No colour ..	Nontoxic ..	0—3	No colour ..	Nontoxic
3—6	...do ...	...do ...	3—6	...do ...	...do ...
6—9	Slight yellow green ...	50	6—9	Slight yellow green	50
9—12	Intense green	15	9—12	...do ...	50
12—16	...do ...	15	12—16	Intense green ...	15

The results in Table 1 refer to the tree analysed immediately after felling: The amount of toxic principles in this tree does not vary appreciably, being very uniform throughout the cross section of the heart wood. The results in Table 2 refer to the tree which had been in the mill pond for some time. In this case the highest toxicity was found in the heart wood adjoining the sap-wood, decreasing toward the center of the cross section. The center of the heart wood contained no volatile toxic substances. The amount of volatile material present in the heart wood adjoining the sapwood did not vary with the height of the tree.

The results show that the amount of toxic principles in these two trees varies greatly. It is, of course, uncertain whether these differences are due to the different treatment of the trees prior to laboratory investigation or to racial or other causes.

In 1941 Cartwright [4] found variability in resistance to decay of the heart wood of home grown (English) Western red cedar in different parts of the log. That extracts of Thuja plicata inhibits the growth of different fungi has been reported by Sowder [5] and Hubert [6].

EXPERIMENTAL

Isolation of β-thujaplicin (A. B. A.)

Sawdust of heart wood of American grown western red cedar was distilled with steam in a copper cylinder, capacity about 5 kg. The distillation was continued until the distillate no longer gave a green ferric coloration. The first 20 liters of the distillate

were quite cloudy, a very small amount of volatile oil finally settling at the bottom. After separating the volatile oil, the total distillate was made alkaline with a concentrated barium hydroxide solution. The yellow precipitate that formed was filtered off and the alkaline solution then reduced to a volume of about 300 ml in large evaporating dishes over a gas flame. After cooling the yellow precipitate was filtered. The yellow barium salts were combined, decomposed with dilute sulphuric acid and the resulting brown oil extracted with ether. The ether solution was then extracted three times with 5 per cent sodium hydroxide solution. The dark coloured alkaline solution was then saturated with carbon dioxide and the thujaplicins thus liberated extracted with ether and distilled b.p. 145—147°/12 mm. The oil partly crystallised, the crystals being γ-thujaplicin. The remaining liquid part showed a green ferric colour reaction and gave the following analytical figures:

$C_{10}H_{12}O_2$ (164.1)   Calc.   C 73.13   H 7.37
                             Found.   » 72.29, 72.47 » 7.35,   7.46

After several years this oil had partly crystallised. The crystals (β-thujaplicin) were removed by filtration and recrystallised from light petroleum, m.p. 52—52.5°.

$C_{10}H_{12}O_2$ (164.1)   Calc.   C 73.13   H 7.37
                             Found.   » 73.07, 73.27 » 7.42,   7.43

Oxidation of β-thujaplicin

The oxidation was carried out in the same manner as the oxidation of γ-thujaplicin[2], 0.2 g β-thujaplicin yielding 0.567 millimoles of volatile acid (46 % of the theoretical amount). The acid was converted into the p-bromophenacyl ester, m.p. 76—77°, unde-pressed in admixture with authentic isobutyric acid p-bromophenacyl ester.

Catalytic hydrogenation of β-thujaplicin

β-Thujaplicin (2 g) in alcohol was hydrogenated with Adams' platinum oxide catalyst. 1150 ml. hydrogen were absorbed (calculated for 4 moles 1100 ml). The catalyst was filtered off and the alcohol removed under reduced pressure. The residue was distilled, yielding a thick oil of octahydro-β-thujaplicin b.p. 152—153°/12 mm, which did not crystallise.

$C_{10}H_{20}O_2$ (172.2)   Calc.   C 69.69   H 11.73
                             Found.   » 69.74   » 11.69

Oxidation of octahydro-β-thujaplicin

The hydrogenation product (0.87 g) was suspended in water (50 ml) and potassium permanganate (1.6 g) added in small portions. The solution was acidified and the manganese dioxide dissolved with sulphur dioxide. The acid was extracted with ether and the ether solution shaken with sodium hydrogen carbonate solution. The acid was again

liberated with dilute sulphuric acid and transferred into ether. On evaporation of the latter an oil remained. This was converted into its bis-benzylthiuronium salt which, after recrystallisation from alcohol—ethyl acetate, had m. p. 152—153°.

$C_{26}H_{38}O_4N_4S_2$ (534.5) Calc. C 58.38 H 7.19
Found. » 58.08 » 7.14

3-*Isopropylcyclo*hexanone

The oily acid (0.2 g) was converted into its barium salt. On dry distillation this yielded a mobile oil with a smell resembling that of menthone. The oil was divided in two parts and converted into the 2,4-dinitrophenylhydrazone and the semicarbazone.

The 2,4-dinitrophenylhydrazone was recrystallised from alcohol and formed orange red leaflets, m. p. 139—140°.

$C_{15}H_{20}O_4N_4$ (320.2) Calc. N 17.50
Found. » 17.64

The semicarbazone was recrystallised from alcohol m. p. 187—188°.

$C_{10}H_{19}ON_3$ (197.2) Calc. N 21.30
Found. » 21.30

Synthesis of β-*iso*propylpimelic acid

2-Hydroxy-4-*iso*propylbenzoic acid was prepared by the method of Jacobsen [7]. This acid (0.6 g) was dissolved in 75 ml of amyl alcohol. To the boiling solution sodium (5 g) was added. When the sodium had dissolved the amyl alcohol was removed by distillation with steam. The remaining alkaline solution was acidified and again subjected to steam distillation in order to remove any unreacted 2-hydroxy-4-*iso*propylbenzoic acid. The nonvolatile acid was extracted with ether in a continuous extractor. The acid thus obtained, however, still contained appreciable amounts of the starting material. By digestion with small portions of water the β-*iso*propylpimelic acid could be obtained free from starting material which is much less readily soluble in water. The β-*iso*propylpimelic acid thus obtained was an oil and was directly converted into its bis-benzylthiuronium salt. This was recrystallised from alcohol—ethyl acetate, m. p. 152.5—153°. Mix. m.p. with the above salt 152.5—153°.

$C_{26}H_{38}O_4N_4S_2$ (534.5) Calc. C 58.38 H 7.19
Found. » 57.99 » 7.23

Distribution of the toxic principles in western red cedar
(A. B. A.)

Sections of two virgin-growth trees were obtained for this investigation. One tree was obtained from Portland, Oregon. It had been in a mill pond for some time. The other tree was sent to the laboratory from Snoqualmie Falls, Washington, directly after felling and it had not been in a mill pond.

The cross section of the butt of each log about 8 inches thick and another cross section about 15 feet towards the top were used for sampling. A strip about 7 inches wide was cut through the center of each cross section. This piece of wood, from the center of the heart wood to the sapwood, was then cut into five sections of equal length. Each section in turn was cut up into sawdust, well mixed, and a weighed quantity of sawdust thus taken from each section to determine the toxicity of its steam volatile components.

A weighed amount (50 g) of sawdust was put into a 2-liter round bottom flask and steam distilled until 500 ml of distillate were collected. Thus 10 ml of steam distillate represented 1 g of wood. Portions of 100, 50, 25, 15, 10 and 5 ml of steam distillate were tested for their toxicity. When the distillate gave no colour with alcoholic ferric chloride the distillate was found to be nontoxic. The results are given in Tables 1 and 2 (pp. 646 and 647).

SUMMARY

The structure of β-thujaplicin, one of the toxic principles of the heart wood of *Thuja plicata* D. Don grown in U. S. A., has been elucidated. It is 1-*iso*-propyl*cyclo*heptatrien-(2,5,7)-ol-(3)-one(4)   *viz.*   1-*iso*propyl*cyclo*heptatrien--(1,4,6)-ol-(4)-one(3) or β-*iso*propyltropolone.

A short account of the distribution of the toxic principles in the heart wood of two samples of *Thuja plicata* is also given.

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