173. *The Active Principles of Leguminous Fish-poison Plants. Part I. The Properties of* l-α-*Toxicarol isolated from* Derris malaccensis (*Kinta Type*).

By Stanley H. Harper.

The optically active precursor of toxicarol has been obtained by direct crystallisation of an ethereal extract of *Derris malaccensis* (Kinta type). After being freed from a small proportion of sumatrol by the method of Cahn, Phipers, and Boam (1938), the *l*-α-toxicarol was identical in properties with that described by Tattersfield and Martin (1937). It is concluded that the optical data of Cahn, Phipers, and Boam are untrustworthy, and their criticism of Tattersfield and Martin unjustified.

Tattersfield and Martin (*J. Soc. Chem. Ind.*, 1937, **56**, 77 T; *Ann. Appl. Biol.*, 1938, **25**, 411) have described the isolation of an optically active, crystalline precursor of toxicarol from " Sumatra-type " *Derris* resin. Cahn, Phipers, and Boam (J., 1938, 513), utilising seed crystals supplied by Dr. Tattersfield, also have been able to obtain this precursor crystalline, and have shown it to be *l*-α-toxicarol. Their work led them to doubt the purity of Tattersfield and Martin's preparation. Recently an authentic sample of *Derris malaccensis* grown in the Kinta district of Malaya was received. As the extract corresponded in character [high ether extract (21%), low rotenone content (1%), and giving much toxicarol with alkali] to the " Sumatra-type " resin of Cahn and Boam (*J. Soc. Chem. Ind.*, 1935, **54**, 37 T), the opportunity was taken of examining the question of the purity of this toxicarol precursor. When a concentrated ethereal extract of the root was cooled in a refrigerator, crystallisation took place spontaneously in quantity corresponding to 60% by weight of the resin. Crystallisation from ethyl acetate–ethyl alcohol gave the crude toxicarol containing sumatrol described by Cahn, Phipers, and Boam (*loc. cit.*). This was freed from sumatrol by their ether trituration method to give pure *l*-α-toxicarol. It was identical in physical and optical properties with a specimen prepared by Dr. Tattersfield. Moreover, examination of a specimen prepared by Cahn, Phipers, and Boam (for which I am indebted to Dr. Cahn) gave the following results :

	Tattersfield and Martin (*locc. cit.*).	Harper.	Cahn, Phipers, and Boam (*loc. cit.*).	Author's figures for the last.
l-α-Toxicarol–*l*-sumatrol mixture, m. p. 101°, $[\alpha]_D$ in benzene	—	−80°	−68°	—
l-α-Toxicarol, m. p. 102·5°, $[\alpha]_D$ in benzene	−67°	−66	−53	−68°
l-α-Toxicarol, m. p. 102·5°, $[\alpha]_D$ in acetone	—	+59	+69	+58

The rotations are all in 4·00% solution, except those of Cahn, Phipers, and Boam, which are in 5—6% solution (see J., 1938, 737). An examination of the variation of $[\alpha]_D$ with concentration (see p. 815) showed that this could not account for the discrepancies between the third and the fourth columns of the table. From the agreement between the different preparations examined in this laboratory it must be concluded that the three preparations are of substantially the same purity and free from *l*-sumatrol. The polarimetric figures of Cahn, Phipers, and Boam would seem to be untrustworthy. Their preparation is

undoubtedly pure, but the conclusion they drew from their optical data as to the purity of Tattersfield and Martin's preparation would appear to be unjustified.

The racemisation of *l*-α-toxicarol and *l*-α-dihydrotoxicarol in benzene–methyl alcohol by potassium hydroxide has been examined, the mechanism of which has been discussed by Cahn, Phipers, and Boam (J., 1938, 513). Tattersfield and Martin (*Ann. Appl. Biol.*, 1936, **23**, 909), using " Sumatra-type " resin, showed that the reaction velocity was proportional to the amount of methyl alcohol added with the alkali. The same phenomenon has been observed with *l*-α-toxicarol (Fig. 1).

Plotting $\log_{10}([\alpha]_t^{18°} - [\alpha]_{t=\infty}^{18°})$ against time (Fig. 2) gave for each experiment a straight line over the first two-thirds of the reaction, indicating that it is essentially unimolecular. The increase of the reaction constant is more rapid than the increase in the proportion of the methyl alcohol, which is due probably to the racemisation occurring in the alcohol

FIG. 1.

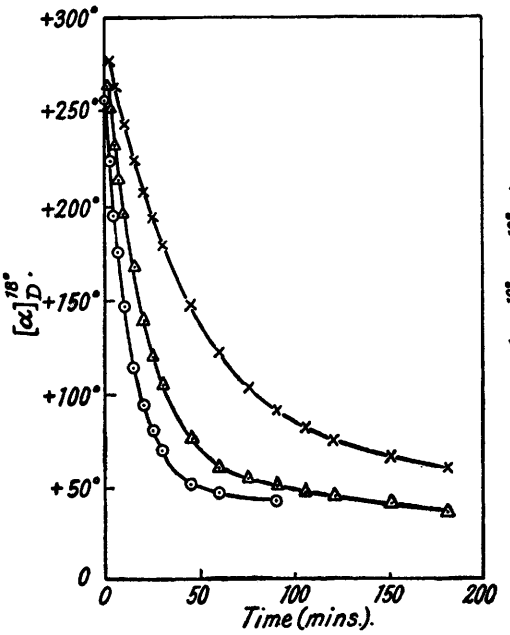
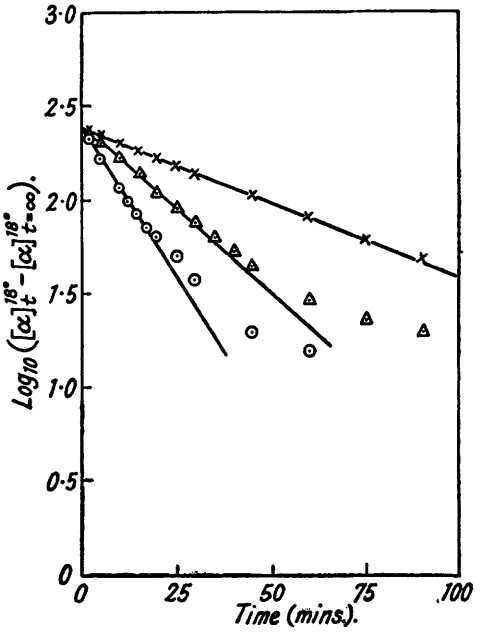


FIG. 2.



0·5 *G. of* l-α-*toxicarol in* 10 *c.c. of benzene with* 1 *equivalent of potassium hydroxide in*

(a) 4 *c.c. of* MeOH, ×———×, k = 0·008.
(b) 8 *c.c. of* MeOH, △———△, k = 0·017.
(c) 12 *c.c. of* MeOH, ⊙———⊙, k = 0·031.

rather than in the benzene, as with alcohol alone the reaction is too rapid to be followed with accuracy. The slowness of the reaction in benzene may be correlated with the observation that *l*-α-toxicarol is not extracted from a benzene solution by aqueous potassium hydroxide, though readily so from ethereal solution.

Rowaan and van Duuren have recently stated (*Chem. Weekblad*, 1938, **35**, 755) that toxicarol occurs in *Derris* extract as protoxicarol; from its properties (lævorotatory in benzene and dextrorotatory in acetone) it is undoubtedly *l*-α-toxicarol.

EXPERIMENTAL.

The *D. malaccensis* used was received in the form of short lengths of air-dried root, which were chopped and finely ground. On analysis the ground root gave 20·9% of ethereal extract and 1·3% of rotenone on an air-dry basis. (The method of rotenone estimation used is to be published elsewhere shortly.) Microanalyses are by Drs. Weiler and Strauss, Oxford. Methoxyl determinations, except where otherwise stated, are by the author, using Clark's semimicro-

method (*J. Assoc. Off. Agric. Chem.*, 1932, **15**, 136). The calibration of the polarimeter used was checked with solutions of pure rotenone in benzene, any differences from the figures of Jones and Smith (*J. Amer. Chem. Soc.*, 1932, **52**, 2557) being within the error of reading the scale. Melting points are uncorrected.

The finely ground air-dried root (1000 g.) was extracted to completion with ether in a large Soxhlet apparatus. The solution was filtered from a little amorphous material and concentrated to 700 c.c. On cooling in a refrigerator, rapid separation of the crude *l*-α-toxicarol took place without the necessity for seeding. Crystallisation proceeded through a gelatinous form, which after a few hours changed into hard yellow nodules. The filtrate from this crop, after concentration, slowly deposited, on prolonged cooling, a further quantity of crude toxicarol. The total yield was 117 g. (*i.e.*, 56% of the resin) (crop A).

The dark red ethereal filtrate from which no more toxicarol would separate was extracted with alkali by method (c) of Cahn, Phipers, and Boam. (J., 1938, 531). The first extracts of emulsifying agents were discarded, only the fractions giving a yellow precipitate being acidified and the precipitated phenols removed in ether. This solution was dried with sodium sulphate, concentrated to small bulk, and kept in a refrigerator for a week, during which yellow crystals separated (9·5 g.) (crop B). The filtrate from this slowly deposited a mixed crop of yellow and colourless plates; the latter were separated by hand and shown to be nearly pure *l*-sumatrol by m. p. (194°) and non-depression of m. p. on admixture with authentic *l*-sumatrol.

Crop A.—This was crystallised from ethyl acetate–ethyl alcohol (1 : 3), and the toxicarol obtained in clusters of long, thin, yellow prisms (85 g.), m. p. 101—102°, $[\alpha]_D^{20°}$ — 80° in 4·00% benzene solution. Cahn, Phipers, and Boam (*loc. cit.*) record $[\alpha]_D$ — 68° in benzene for a similar preparation.

A portion of this was freed from *l*-sumatrol by the above authors' method of trituration with ether, and crystallised from ethyl acetate–ethyl alcohol repeatedly until a further crystallisation changed neither the m. p. nor the optical rotation. The pure *l*-α-toxicarol so obtained formed yellow plates, m. p. 102·5°, $[\alpha]_D^{20°}$ — 66° in 4·00% benzene solution, $[\alpha]_D^{20°}$ + 59° in 4·00% acetone solution. Cahn, Phipers, and Boam (*loc. cit.*) record — 53° and + 69° respectively.

As the proportion of contaminating sumatrol was small, the somewhat laborious purification of Cahn, Phipers, and Boam was simplified as follows: 45·0 g. of powdered toxicarol (m. p. 101—102°, $[\alpha]_D^{20°}$ — 80° in benzene) were mechanically shaken with 450 c.c. of ether for 2 hours. The undissolved, practically white solid (5·4 g.) was fairly pure *l*-sumatrol, m. p. 165—170°. The filtrate was concentrated to 150 c.c. and kept in a refrigerator, the toxicarol crystallising in masses of yellow prisms (34·3 g.). This crop was reshaken with ether (10 vols.), and the insoluble portion examined for the presence of sumatrol by further trituration with ether; only toxicarol, however, was present. The ethereal solutions were therefore concentrated and allowed to crystallise, and the crops of toxicarol combined (29·1 g.), m. p. 95°. Two crystallisations from ethyl acetate–ethyl alcohol (1 : 3) sufficed to obtain constancy of optical rotation, giving *l*-α-toxicarol (23·0 g.) identical in properties with that prepared above, m. p. 102·5°, $[\alpha]_D^{20°}$ — 66° in 4·00% benzene solution. It was found advantageous in later preparations to insert a crystallisation from ethyl acetate (2 vols.) after the ether trituration. The crude *l*-α-toxicarol ($[\alpha]_D$ — 80°) contained approximately 10% of *l*-sumatrol.

Analyses of this purified *l*-α-toxicarol were very difficult to interpret (Found: C, 66·5, 66·4; H, 5·5, 5·5. Calc. for $C_{23}H_{22}O_7$: C, 67·3; H, 5·4%). Cahn, Phipers, and Boam (J., 1938, 522) had obtained similar values for carbon of 0·5 — 1·4% low for toxicarol on material containing sumatrol. This divergence from the expected figures has been found to extend to the methoxyl content [Found: (semimicro) MeO (preparations by the author, air-dried), 15·85, 15·8, 15·8; (after 8 hours on the Hyvac pump at 85°), 15·6; (another preparation dried in a vacuum at room temperature), 15·6; (preparation by Dr. Tattersfield, air-dried), 15·9; (preparation by Cahn, Phipers, and Boam, air-dried), 15·9; (micro) MeO (dried in a vacuum at room temperature), 15·8. Calc. for $C_{23}H_{22}O_7$: MeO, 15·1%]. No difficulty was experienced in analysing either rotenone (Found: MeO, 15·8, 15·8. Calc. for $C_{23}H_{22}O_6$: MeO, 15·7%) or *dl*-α-toxicarol (Found: MeO, 15·0. Calc. for $C_{23}H_{22}O_7$: MeO, 15·1%). The formula $C_{22}H_{22}O_7$ (requires C, 66·3; H, 5·6; MeO, 15·6%) fits the above data quite well, yet it is difficult to reconcile this with the ready formation of *dl*-α-toxicarol ($C_{23}H_{22}O_7$) by mild alkali treatment and of *l*-dihydrotoxicarol ($C_{23}H_{24}O_7$) by catalytic hydrogenation (see below).

Crop B.—Two crystallisations from ethyl acetate–ethyl alcohol gave *l*-α-toxicarol (5·1 g.) in clusters of yellow plates, m. p. 100°, $[\alpha]_D$ — 64° in 4·00% benzene solution. There was no indication of the presence of *l*-β-toxicarol.

Apart from the alkali-soluble emulsifying agents, *l*-α-toxicarol and *l*-sumatrol appear to constitute the whole of the phenolic portion of *D. malaccensis* (Kinta type) resin.

Variation of $[\alpha]_D$ *with Concentration.*—In an attempt to elucidate the disagreement of $[\alpha]_D$ between that recorded by Cahn, Phipers, and Boam (*loc. cit.*) and that found by the author, the variation of $[\alpha]_D$ with concentration was determined :

Concn. % in benzene	2·00	4·00	5·00	6·00	8·00	10·00
$[\alpha]_D^{20°}$	−69·1°	−66·4°	−64·2°	−62·0°	−60·0°	−57·5°

The variation is approximately linear, and as such is given by the equation $[\alpha]_D^{20°} = 1{\cdot}46c - 72{\cdot}0°$, where c is the % concentration of *l*-α-toxicarol in benzene. Calculation from the equation gives the following :

Concn. % in benzene	2·00	4·00	5·00	6·00	8·00	10·00
$[\alpha]_D^{20°}$	−69·1°	−66·2°	−64·7°	−63·2°	−60·3°	−57·4°

It was found difficult to replicate rotations in acetone solution with different preparations, though these were constant in benzene solution. This may be due to differences in the purity of the acetone used. The variation of $[\alpha]_D$ with concentration was small and barely significant :

Concn. % in acetone	4·00	6·00	8·00
$[\alpha]_D^{20°}$	+59°	+58°	+57°

It is evident that the variation in concentration used (5—6%) by the above authors does not account for the differences found.

Equilibration of l-α-*Toxicarol.*—(a) To *l*-α-toxicarol (0·5 g.) in benzene (10 c.c.), 1·7% methyl-alcoholic potassium hydroxide (4 c.c.; 1 equiv.) was added. The following rotations were observed in a 1 dm. tube when the solution was kept :

Time, mins.	2	5	7·5	10	15	20	25	30	45
$[\alpha]_D^{18°}$	+276°	+261°	+250°	+241°	+223°	+206°	+192°	+178°	+146°
Time, mins.	60	75	90	105	120	150	180	270	400
$[\alpha]_D^{18°}$	+121°	+102°	+ 90°	+ 81°	+ 74°	+ 66°	+ 59°	+ 49°	+ 45°

(b) The quantities of (a) with an additional 4 c.c. of methyl alcohol added :

Time, mins.	1	2	3	5	7·5	10	15	20	25	30
$[\alpha]_D^{18°}$	+293°	+263°	+250°	+231°	+213°	+195°	+167°	+138°	+119°	+104°
Time, mins.	35	40	45	60	75	90	105	120	150	180
$[\alpha]_D^{18°}$	+ 93°	+ 82°	+ 75°	+ 59°	+ 53°	+ 50°	+ 46°	+ 44°	+ 40°	+ 36°

(c) The quantities of (a) with an additional 8 c.c. of methyl alcohol added :

Time, mins.	<1	1	2	3	4	5	6	7	8	9
$[\alpha]_D^{18°}$	+279°	+255°	+239°	+223°	+209°	+194°	+186°	+174°	+165°	+157°
Time, mins.	10	12·5	15	17·5	20	25	30	45	60	90
$[\alpha]_D^{18°}$	+145°	+128°	+113°	+100°	+ 93°	+ 79°	+ 68°	+ 50°	+ 46°	+ 42°

These results are plotted in Figs. 1 and 2.

The racemisation was accompanied by side reactions productive of red impurities which finally rendered the solution too opaque to read. In no experiment, however, was a fall to zero observed as claimed by Cahn, Phipers, and Boam under the same conditions.

No crystalline product could be obtained in attempted acetylation of *l*-α-toxicarol by (a) acetic anhydride in pyridine at room temperature, or (b) boiling acetic anhydride for short periods.

Methylation by diazomethane in benzene–ether was unsuccessful, as only *l*-α-toxicarol could be isolated in poor recovery.

l-α-*Dihydrotoxicarol.*—Pure *l*-α-toxicarol (20·0 g.) and a platinum oxide catalyst (0·5 g.) in purified dioxan (250 c.c.) (Oxford, *Biochem. J.*, 1934, **28**, 1328) were stirred under hydrogen until absorption ceased. *l*-α-Dihydrotoxicarol was isolated by pouring the filtered solution into water and crystallising the precipitate from alcohol. Despite treatment with charcoal and repeated crystallisation the *l*-α-dihydrotoxicarol (13·9 g.; 70% of the theoretical yield) formed pale yellow needles, m. p. 173—174°, $[\alpha]_D^{19°}$ − 37° in 5·00% benzene solution (Found : C, 66·8; H, 5·8; MeO, 15·0. Calc. for $C_{23}H_{24}O_7$: C, 67·0; H, 5·9; MeO, 15·0%). Cahn, Phipers, and Boam (J., 1938, 534) record m. p. 178—180° and $[\alpha]_D$ − 57° in benzene.

Reduction in " AnalaR " acetic acid at room temperature gave a product similar in properties and yield.

l-α-*Dihydrotoxicarol Monoacetate.*—*l*-α-Dihydrotoxicarol (5·0 g.), anhydrous sodium acetate (2·0 g.), and acetic anhydride (50 c.c.) were boiled under reflux for 10 minutes and then poured into water. The residue after destruction of the anhydride crystallised from alcohol in colourless needles (3·5 g.), m. p. 179°, $[\alpha]_D^{18°}$ + 57° in 5·00% acetone solution (Found: C, 65·7; H, 5·65; MeO, 13·65. Calc. for $C_{25}H_{26}O_8$: C, 66·1; H, 5·8; MeO, 13·65%). Cahn, Phipers, and Boam (*loc. cit.*) record m. p. 184—186°, $[\alpha]_D$ + 64·5° in acetone.

Hydrolysis of the acetate by hot 5% alcoholic hydrochloric acid for 30 minutes gave the parent phenol, m. p. 170° (after softening at 167°), $[\alpha]_D^{18°}$ − 31° in 5·00% benzene solution, indicating that partial racemisation had occurred.

Attempted methylation of the parent phenol with diazomethane in benzene–ether was unsuccessful, *l*-α-dihydrotoxicarol being obtained in 80% recovery.

Equilibration of l-α-*Dihydrotoxicarol.*—To *l*-α-dihydrotoxicarol (0·5 g.) in benzene (10 c.c.), 1·7% methyl-alcoholic potassium hydroxide (4 c.c.; 1 equiv.) was added. The following rotations were observed:

Time, mins.	1	5	10	15	30	45	60	75
$[\alpha]_D^{18°}$	+259°	+244°	+229°	+210°	+168°	+133°	+113°	+ 94°
Time, mins.	90	105	120	150	180	210	240	1440
$[\alpha]_D^{18°}$	+ 83°	+ 72°	+ 64°	+ 54°	+ 49°	+ 43°	+ 41°	—

A reading could not be made after 24 hours owing to the separation of impure *dl*-dihydrotoxicarol in reddish-orange needles. A reddening, though not so pronounced as with *l*-α-toxicarol, was observed. Log_{10} ($[\alpha]_D^{18°}$ − 40°) plotted against time gave a straight line over the period 0 to 180 minutes, with $k = 0{\cdot}008$, indicative of a unimolecular reaction.

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