221. *The Active Principles of Leguminous Fish-poison Plants. Part V.* Derris malaccensis *and* Tephrosia toxicaria.

By Stanley H. Harper.

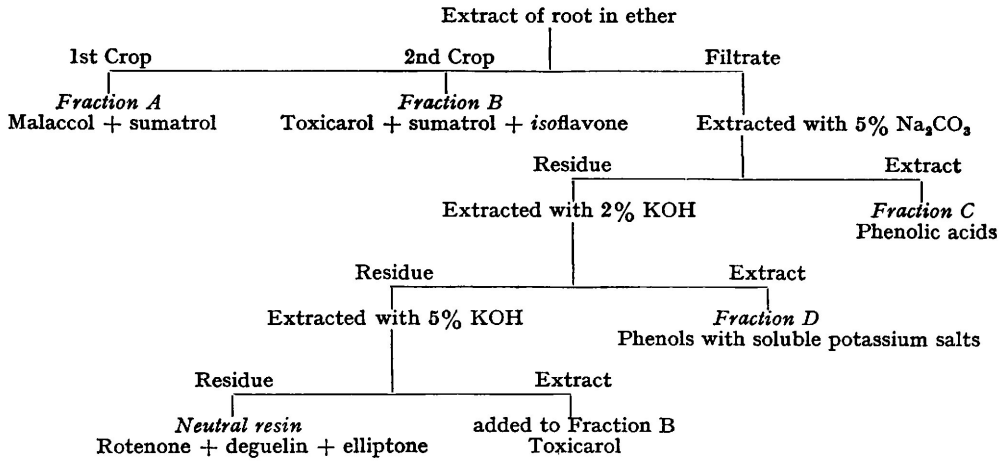
The resin from *D. malaccensis* root has been fractionated by chemical means, and *l*-α-toxicarol obtained in a pure condition. In addition rotenone, elliptone, deguelin, malaccol, sumatrol, and a new phenol have been isolated. The properties of this

phenol, which is isomeric with toxicarol, are discussed and as a working hypothesis an *iso*flavone structure (IV) is suggested.

The resin from *T. toxicaria* root has been similarly fractionated, and rotenone, *l*-α-toxicarol, and sumatrol isolated.

In Part I (J., 1939, 812) the isolation of *l*-α-toxicarol from *Derris malaccensis* root and its separation from sumatrol was examined, the properties of the substance agreeing with those recorded by other workers (Tattersfield and Martin, *J. Soc. Chem. Ind.*, 1937, **56**, 77T; Cahn, Phipers, and Boam, J., 1938, 513). It was pointed out, however, that it did not behave on analysis as a pure substance, particularly in view of its high methoxyl content, and the suggestion was made (*Chem. and Ind.*, 1938, **57**, 451) that a third substance might be present. Cahn, Phipers, and Boam (*loc. cit.*) too had suggested the possibility of a third substance, other than sumatrol, in the crude product. Subsequently malaccol was isolated in small yield from this root (Meyer and Koolhaas, *Rec. Trav. chim.*, 1939, **58**, 207; Harper, this vol., p. 309), although separating in a different fraction to the toxicarol, and it was uncertain to what extent this affected the previous findings. From the experience gained and the material accumulated in the work on malaccol it has been possible to re-examine successfully the question of the homogeneity of *l*-α-toxicarol.

The ethereal extract of the root was therefore fractionated according to the following scheme :



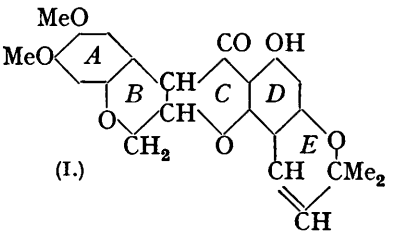
The first precipitate, which was gelatinous and separated before the crystalline toxicarol, was shown (this vol., p. 309) to contain malaccol, and the acetone filtrate from this has deposited only sumatrol on keeping for as long as six months in the refrigerator. The second precipitate of crude toxicarol constituting the main bulk of the resin was examined in detail as described below. When on concentration of the ethereal solution and refrigeration no further toxicarol would separate, the solution was extracted first with sodium carbonate to give a fraction of phenolic acids and secondly with 2% potassium hydroxide solution, which separated a phenolic fraction. This was distinct from toxicarol because it gave a soluble potassium salt and failed to crystallise from ether; these fractions were not, however, examined in detail. Subsequent extraction with 5% potassium hydroxide solution gave the characteristic insoluble yellow salt of toxicarol, which was acidified under ether, and the toxicarol that crystallised added to that obtained previously. The ethereal extract then gave on evaporation the neutral resin, which, by the method elaborated for the separation of elliptone (J., 1939, 1099), was shown to contain rotenone, deguelin, and elliptone. Neither of the last two has previously been reported as occurring in *D. malaccensis*. An account of this is, however, reserved for a subsequent communication.

The crude toxicarol was first fractionated from ethyl acetate solution. After ten crystallisations a series of head fractions of toxicarol of constant specific rotation ($[\alpha]_D^{20°}$ — 67° in benzene) were taken off until no more would separate. This material, although

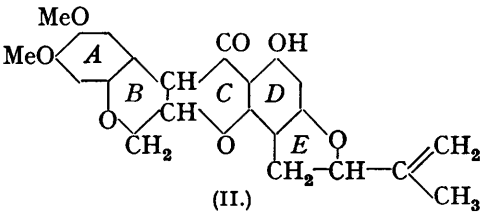
bright yellow and devoid of the greenish tinge reported previously (Cahn, Phipers, and Boam, *loc. cit.*), was expected to contain sumatrol. These head fractions were therefore fractionated from ether by the method described previously (J., 1939, 812). The fractions obtained, however, had the same specific rotation and both sumatrol and malaccol, which is also sparingly soluble in ether, were absent. This material was thus homogeneous and therefore pure *l*-α-toxicarol. The pure *l*-α-toxicarol so obtained, giving the correct analysis for $C_{23}H_{22}O_7$, crystallised from ether and ethyl acetate–alcohol in bright yellow laths in an indefinitely solvated condition, m. p. 100° and 103° respectively, but from light petroleum in unsolvated yellow prisms, m. p. 127°. It is characterised by a marked retentivity of solvent, which in the case of ether is only removed by fusion in a vacuum. No doubt is felt as to the purity of this material, because the method of fractionation with these two solvents would quickly remove impurities, toxicarol going first to the head and then to the tail of the crystallisations. Its analysis is correct and moreover it gives derivatives in an optically pure condition without recrystallisation. Catalytic hydrogenation gave *l*-dihydrotoxicarol, m. p. 179°, $[\alpha]_D^{20°}$ — 30° in benzene, properties which were unchanged after regeneration of the substance from the twice crystallised acetate. Racemisation by sodium acetate–alcohol gave a high yield of *dl*-α-toxicarol, m. p. 219°, giving no colour in the Goodhue test and therefore free from β-toxicarol. The author has obtained similar material by using, on earlier samples of toxicarol, the purification method of Cahn, Phipers, and Boam (*loc. cit.*), whereas they themselves report a melting point of 233° and state that *dl*-α-toxicarol of m. p. 219° contains 4·5% of β-toxicarol. This discrepancy cannot be accounted for. It is noteworthy that Jones [*Ind. Eng. Chem.* (*Anal. Edit.*), 1939, **11**, 429], in preparing *dl*-α-toxicarol by the above authors' method, was unable to raise the m. p. above 217° (corr.), though his material still contained a trace of β-toxicarol, and that Clark (*J. Amer. Chem. Soc.*, 1930, **52**, 2461), who first isolated *dl*-α-toxicarol, reported 219° (corr.).

The ethyl acetate mother-liquors from the toxicarol fractionation gave on concentration a small crop of a *substance* closely simulating toxicarol. By repeated crystallisation from ethyl acetate it was obtained in pale yellow needles melting at 219°. Like *dl*-α-toxicarol, it was optically inactive and phenolic, giving a deep green colour with alcoholic ferric chloride; a mixture, however, gave a well-marked depression of melting point. Their non-identity was further shown by the negative Durham test given by this substance and a methoxyl content of 22·6% as against 15·1% for toxicarol. Elementary analysis, coupled with the methoxyl content, established that the substance has the formula $C_{23}H_{22}O_7$ and is thus isomeric with toxicarol and sumatrol, but has three instead of two methoxyl groups per molecule. Analyses of derivatives described below are in accord with this formula. This substance readily forms solid solutions with *l*-α-toxicarol and it is therefore probable that, coupled with the retention of solvent, it accounts for the high methoxyl contents recorded previously for *l*-α-toxicarol.

It has not been possible in present circumstances to make a complete examination of this substance, but with the material available some of its reactions have been studied. From earlier work a positive Durham test would seem to be specific for the chromenochromanone ring structure *A*, *B*, *C*, and *D* as in toxicarol (I) and sumatrol (II), which is therefore modified or absent in this substance. Moreover it is impossible to formulate a structure of this type containing an additional methoxyl group which yet remains isomeric with toxicarol and sumatrol. Acetylation and benzoylation give respectively O-*monoacetyl* and



(I.)



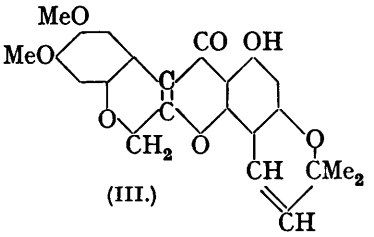
(II.)

O-*monobenzoyl* derivatives, giving no colour with ferric chloride, and so establishing the presence of one phenolic hydroxyl group. The strong ferric chloride reaction, coupled

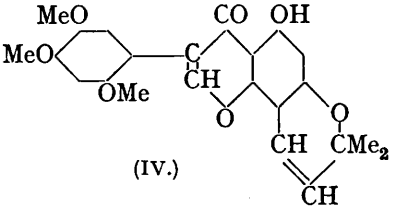
with the insolubility in alkali, suggests the presence of a keto-group ortho to the hydroxyl group, as in toxicarol (I) and sumatrol (II).

Attempts to establish the presence of a keto-group by oximation were unsuccessful, but in the light of the difficulty of oximating toxicarol (George and Robertson, J., 1937, 1535) and of sumatrol (Robertson and Rusby, *ibid.*, p. 497) this cannot be regarded as excluding its presence. If present, it must be incapable of enolisation, as under the conditions used for monoacetylation and monobenzoylation toxicarol gives in addition to mono-derivatives diacetyl- and dibenzoyl-toxicarol through enolisation of the keto-group. This substance is therefore more akin to dehydrotoxicarol (III), which, being incapable of enolisation, gives only a monoacetyl derivative and moreover a negative Durham test. Methylation of this substance with methyl sulphate in potassium carbonate–acetone, giving a *monomethyl ether*, further distinguishes it from toxicarol, which under similar conditions suffers fission of ring *C* to give an *O*-dimethyl derivative (Cahn, Phipers, and Boam, J., 1938, 734). The stability of this substance to boiling alcoholic sulphuric acid precludes its formulation as an ether of the type of the rotenolone methyl ethers, for these lose methyl alcohol under such conditions (LaForge and Haller, *J. Amer. Chem. Soc.*, 1934, **56**, 1620). It does not, however, exclude the presence of an *iso*propenyl furan ring, since, although rotenone is readily isomerised to *iso*rotenone in the presence of acid, sumatrol is recovered unchanged (Robertson and Rusby, *loc. cit.*).

It is not possible from this evidence to establish a formula with any degree of certainty; nevertheless, occurring with toxicarol and sumatrol, the substance is likely to be closely related to them. Therefore as a working hypothesis the *iso*flavone structure (IV) is suggested.



(III.)



(IV.)

The isolation of this substance in an optically inactive form by direct crystallisation suggests its presence in the resin as such. Preference is therefore given to the presence of a 2 : 2-dimethyl-Δ^3-chromen ring as in toxicarol instead of the *iso*propenyl furan ring of sumatrol with its asymmetric carbon atom. The *iso*flavone structure is preferred to that of a flavone, which also is consistent with the data, owing to the closer relationship of the former to toxicarol. Such a formula, if substantiated, is of great biochemical interest as suggesting a link in the biogenesis of toxicarol in the plant. Moreover the possibility is presented of there being a series of *iso*flavones in the plant corresponding to rotenone, etc., and similarly constituted.

dl-α-Toxicarol was isolated by Clark (*Science*, 1930, **71**, 396; *J. Amer. Chem. Soc.*, 1930, **52**, 2461) from the roots of *Tephrosia toxicaria* previous to its isolation from *D. malaccensis* (Spoon, *De Indische Mercuur*, 1932, **55**, 181). Subsequently the isolation of *l*-α-toxicarol from the latter showed that toxicarol was present in the root as the optically active form. It therefore seemed probable that toxicarol was present as *l*-α-toxicarol in *T. toxicaria*. This point has now been elucidated, roots of *T. toxicaria* from both Malaya and British Guiana being used. To facilitate crystallisation, the phenols were separated through their potassium salts and crystallised from ether. After seeding and prolonged refrigeration crude optically active toxicarol separated, which by the ether trituration method (J., 1939, 812) was separated into sumatrol and *l*-α-toxicarol, though the small quantities prevented complete purification. This is the first recorded instance of the occurrence of sumatrol other than in *Derris* root and suggests that it generally accompanies toxicarol. Rotenone was readily separated by crystallisation of the neutral resin from carbon tetrachloride, though its presence in this species has not previously been recorded. It is known, however, to occur in several other species of *Tephrosia*. Subsequent to the completion of this work Castagne (Contribution a l'Étude Chimique des Légumineuses

Insecticides du Congo Belge, Brussels, 1938) has reported the isolation of rotenone from this species, but failed to isolate optically active toxicarol.

Experimental.

Microanalyses are by Drs. Weiler and Strauss, Oxford. Methoxyl determinations are by the author, using Clark's semimicro-method (*J. Assoc. Off. Agric. Chem.*, **1932, 15**, 136). Melting points were observed in Mason's apparatus (*Chem. and Ind.*, 1925, 577) and are uncorrected.

A number of extractions and fractionations were carried out and this is a typical experiment. The finely ground air-dried root (1320 g.) was extracted to completion with ether in a large Soxhlet apparatus. The extract (1·5 l.), on standing overnight, deposited a gelatinous precipitate (fraction A), which was filtered off and air-dried (25 g.). The filtrate on refrigeration rapidly deposited hard crystalline masses of crude toxicarol. A further quantity could be obtained by concentrating and keeping the filtrate (131 g., fraction B). The ethereal solution was then washed with successive portions of 5% sodium carbonate solution until nothing further was extracted; acidification of these gave fraction C (12 g.). A similar washing with 2% potassium hydroxide solution, acidification, and recovery through ether gave fraction D (14 g.). The ethereal solution was finally extracted with 500 c.c. of 5% potassium hydroxide solution, giving a copious precipitate of the yellow potassium salt of toxicarol, which was recovered by acidification under ether and crystallisation (25 g., added to fraction B). The remaining neutral ethereal extract was washed with acid and water and evaporated, and the residue heated on the steam-bath in a vacuum for 30 mins. to give the neutral resin (35 g.). The examination of this resin will be reported in a forthcoming communication.

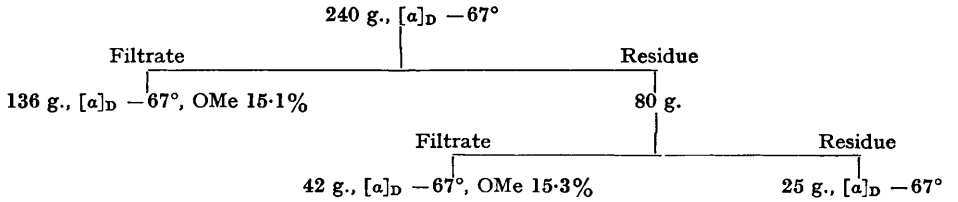
Fraction A. The examination of this fraction was reported recently (this vol., p. 309), when it was shown to contain malaccol and sumatrol. Further crystallisation of the mother-liquors has led only to sumatrol.

Fraction C. This acid fraction was obtained by filtration and drying in a vacuum as a red resin, $[\alpha]_D$ + 18° (approx.) in acetone, soluble in ethyl acetate and acetone, but not in hydrocarbon solvents (Found: OMe, 6·5%). With alcoholic ferric chloride it gave an intense red-brown colour and in the Durham test only a very faint green.

Fraction D. This fraction, obtained as a red resin, gave a deep green colour with alcoholic ferric chloride; it had $[\alpha]_D$ − 31° in benzene (*c*, 1·354; *l*, 1) (Found: OMe, 12·6%). A solution in ether on keeping deposited a small crop of yellow dehydro-compounds and the resin on recovery gave a negative Durham test. It could not be obtained crystalline and so was not further examined. This fraction is quite distinct from the phenols soluble in 5% potassium hydroxide solution in giving a soluble potassium salt.

Fraction B. These were bulked from several separations. The crude toxicarol (900 g.) was divided into six lots and crystallised at 0° from 2 vols. of ethyl acetate; further crystallisations separated the material roughly into fractions of differing solubility, which were then subjected to a rigorous fractional crystallisation. After ten crystallisations the head fraction had reached constant specific rotation, $[\alpha]_D$ − 67° in benzene (*c*, 5·00; *l*, 1),* and a series of head fractions with this rotation were taken off (250 g., fraction E). The ethyl acetate solutions were then bulked and concentrated; no further toxicarol separated, but a small crop of material, obviously not toxicarol though with m. p. 100—105°, was obtained (2 g., fraction F). The ethyl acetate solution was evaporated, and the resin dissolved in ether (2 l.) and washed with 500 c.c. of 2% potassium hydroxide solution. After drying, the ethereal solution was refrigerated, crude toxicarol separating rapidly. A further crop was obtained by concentration (302 g., fraction G). The filtrate has not so far deposited any further crystals.

Fraction E. This was fractionated by mechanical shaking with 10 vols. of ether for 30 mins., filtration, and concentration of the filtrate to one-third of its bulk for crystallisation. The insoluble residue was re-treated with ether (cf. J., 1939, 812) as follows:



* All samples of toxicarol, unless otherwise stated, were fused in a vacuum at 110° for 30 mins. before analysis and determination of $[\alpha]_D$ in 5% benzene solution.

The major most soluble fraction was therefore unchanged and is undoubtedly pure *l*-α-toxicarol. As a check a 20 g. portion was re-treated with ether, giving from the filtrate a crop (11·5 g., $[\alpha]_D$ — 67·4°, OMe 15·2%), and a residue (6·0 g., $[\alpha]_D$ — 67·4°, OMe 15·0%). The material was therefore homogeneous.

l-α-Toxicarol crystallised from ether in bright yellow laths, m. p. 100° with evolution of solvent. The crystals contained ether, which was only slowly lost on drying but did not correspond to any simple solvate [Found : OMe (air-dried material), 17·0; (material dried in a vacuum at 75° for 6 hrs.), 16·3; (material fused in a vacuum) C, 67·4; H, 5·6; OMe, 15·1. Calc. for $C_{23}H_{22}O_7$: C, 67·3; H, 5·4; OMe, 15·1%]. From ethyl acetate–alcohol it crystallised in a similarly solvated condition in bright yellow laths, m. p. 103° [Found : OMe (material dried in a vacuum at 75° for 6 hrs.), 16·0%]. However, from light petroleum (b. p. 60—80°) *l*-α-toxicarol crystallised in unsolvated yellow prisms, m. p. 127°, $[\alpha]_D^{20°}$ — 67° in benzene, + 37° in chloroform, and + 61° in acetone (*c*, 5·00; *l*, 1) [Found : OMe (air-dried material), 15·1%]. It gave no colour in the Goodhuetest (*J. Assoc. Off. Agric. Chem.*, 1936, **19**, 118).

l-*Dihydrotoxicarol.*—Reduction of *l*-α-toxicarol as described previously (J., 1939, 815) gave *l*-dihydrotoxicarol in pale yellow needles, m. p. 179°, $[\alpha]_D^{20°}$ — 30° in benzene (*c*, 5·00; *l*, 1) (Found : OMe, 15·1. Calc. for $C_{23}H_{24}O_7$: OMe, 15·0%). Acetylation (*loc. cit.*) gave the *O*-acetyl derivative in colourless needles, m. p. 184°, $[\alpha]_D^{20°}$ + 59° in acetone (*c*, 5·06; *l*, 1) (Found : OMe, 13·6. Calc. for $C_{25}H_{26}O_8$: OMe, 13·6%). Cahn, Phipers, and Boam (J., 1938, 534) have stated that this compound can be hydrolysed by boiling with 5% alcoholic hydrochloric acid for 30 mins.; repetition of this, however, led to mainly unhydrolysed material. Finally the compound was refluxed with 5% alcoholic sulphuric acid for 6 hrs.; on cooling, *l*-dihydrotoxicarol of the same m. p. and rotation as above separated, unaltered by further crystallisation.

dl-α-*Toxicarol.*—*l*-α-Toxicarol (5 g.) and sodium acetate (10 g., anhydrous) were refluxed for 2 hrs. in ethyl alcohol (100 c.c.). The liquid was filtered hot, and the solid washed with alcohol and hot water, to give *dl*-α-toxicarol (4·0 g.), m. p. 219°. The m. p. was not raised by crystallisation from acetic acid (Found : OMe, 15·0%). The product gave no colour in the Goodhue test and was therefore free from β-toxicarol.

Fraction F.—After four crystallisations from ethyl acetate, in which it was sparingly soluble, this fraction gave a *substance* in pale yellow needles, m. p. 219°, $\alpha_D \pm 0°$ in chloroform. In the Durham test it gave no colour, but with alcoholic ferric chloride a deep green colour and with concentrated sulphuric acid a deep orange non-fluorescent solution were obtained. Before analysis this and its derivatives were dried in a vacuum at 100° for 1 hour (Found : C, 67·3; H, 5·45; OMe, 22·6. $C_{23}H_{22}O_7$ requires C, 67·3; H, 5·4; 3OMe, 22·7%).

O-*Monoacetyl derivative.* The substance (98 mg.) was refluxed in acetic anhydride (1 c.c.) and pyridine (0·5 c.c.) for 1 hour and poured into water. The precipitate was crystallised from alcohol to give the *monoacetate* in colourless needles, m. p. 210° (Found : C, 66·1; H, 5·25; OMe, 20·65. $C_{25}H_{24}O_8$ requires C, 66·35; H, 5·3; OMe, 20·6%). It gave no colour with alcoholic ferric chloride.

O-*Monobenzoyl derivative.* The substance (100 mg.) was refluxed with benzoyl chloride (0·2 c.c.) and pyridine (1 c.c.) for 1 hour. After decomposition with water the precipitate was dissolved in chloroform–alcohol and crystallised by evaporation of the chloroform. The *monobenzoate* was obtained in colourless prisms, giving no colour with ferric chloride; m. p. 193° (Found : C, 68·7; H, 5·0; OMe, 18·3. $C_{30}H_{26}O_8$ requires C, 69·9; H, 5·1; OMe, 18·1%).

O-*Monomethyl derivative.* The substance (100 mg.), methyl sulphate (0·5 c.c.), and potassium carbonate (200 mg.) were refluxed for 6 hours in acetone (15 c.c.). Next day the ferric chloride reaction was still positive, so methyl sulphate (0·5 c.c.) and potassium carbonate (200 mg.) were added and refluxing continued for another 6 hours; the ferric reaction was then negative. By pouring into water and crystallisation from benzene–light petroleum the *ether* was obtained in colourless prisms (82 mg.), m. p. 178° (Found : C, 66·4; H, 5·4; OMe, 28·0. $C_{24}H_{24}O_7$ requires C, 67·9; H, 5·7; OMe, 29·2%).

Fraction G.—From the analysis ($[\alpha]_D$ — 84°; OMe, 15·2%) this fraction appeared to consist only of a mixture of toxicarol and sumatrol. After two crystallisations from ethyl acetate–alcohol it was subjected to ether trituration (as described for fraction E), giving a residue (30 g.) of nearly pure sumatrol, m. p. 190°. The ethereal filtrates were concentrated, and the crops recrystallised from ethyl acetate–alcohol to give pure toxicarol identical with that prepared above.

Tephrosia toxicaria.—The ground root (1500 g.) (from Malaya) was extracted with ethyl acetate in a large Soxhlet apparatus. The pale red extract was evaporated, and the resin treated

with two 500 c.c. portions of ether, filtering into a separating-funnel. This extract was washed with successive portions of 5% potassium hydroxide solution saturated with sodium chloride. Only those portions were retained that gave a yellow precipitate of toxicarol salt. The neutral ethereal layer was dried and evaporated, and the resin (29 g.) dissolved in carbon tetrachloride (150 c.c.). After seeding and refrigeration, rotenone–carbon tetrachloride solvate (6·0 g.) separated. Recrystallisation from carbon tetrachloride and then from alcohol gave rotenone (3·2 g.; yield, 0·2%, calculated on the weight of root), m. p. 163·5°, undepressed on admixture with an authentic specimen. The alkaline extracts, containing yellow potassium salt, were acidified under ether, and the extract washed, dried, and concentrated to 200 c.c. On standing overnight, dehydro-compounds (0·3 g.) separated; then, on refrigeration over a period of 4 months, the filtrate deposited crude *l*-α-toxicarol (9·2 g.). This was crystallised once from ethyl acetate–alcohol and then triturated with ether. The residue on crystallisation from ethyl acetate gave a small crop of dehydrotoxicarol and on addition of alcohol to the filtrate a crop of sumatrol (0·6 g.) admixed with dehydro-compounds. After separation by hand the sumatrol had m. p. 182°, undepressed on admixture with an authentic specimen. The ether-soluble fraction on concentration gave *l*-α-toxicarol (3·0 g.). Crystallised once from ethyl acetate–alcohol, it had m. p. 98°, $[\alpha]_D$ — 77° in benzene. The m. p. was undepressed by an authentic specimen, but this preparation evidently still contained sumatrol.

Extraction of a sample of *T. toxicaria* from British Guiana gave similar results, both rotenone and toxicarol being isolated.

I am indebted to the Directors, the Departments of Agriculture, Malaya, and British Guiana, for the supply of root, and to the Ministry of Agriculture and the Colonial Development Fund for grants which have made this work possible.

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