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CHEMICAL INVESTIGATIONS ON *PUTRANJIVA ROXBURGHII*

THE STRUCTURE OF A NEW TRITERPENE, PUTRANJIVADIONE[1]

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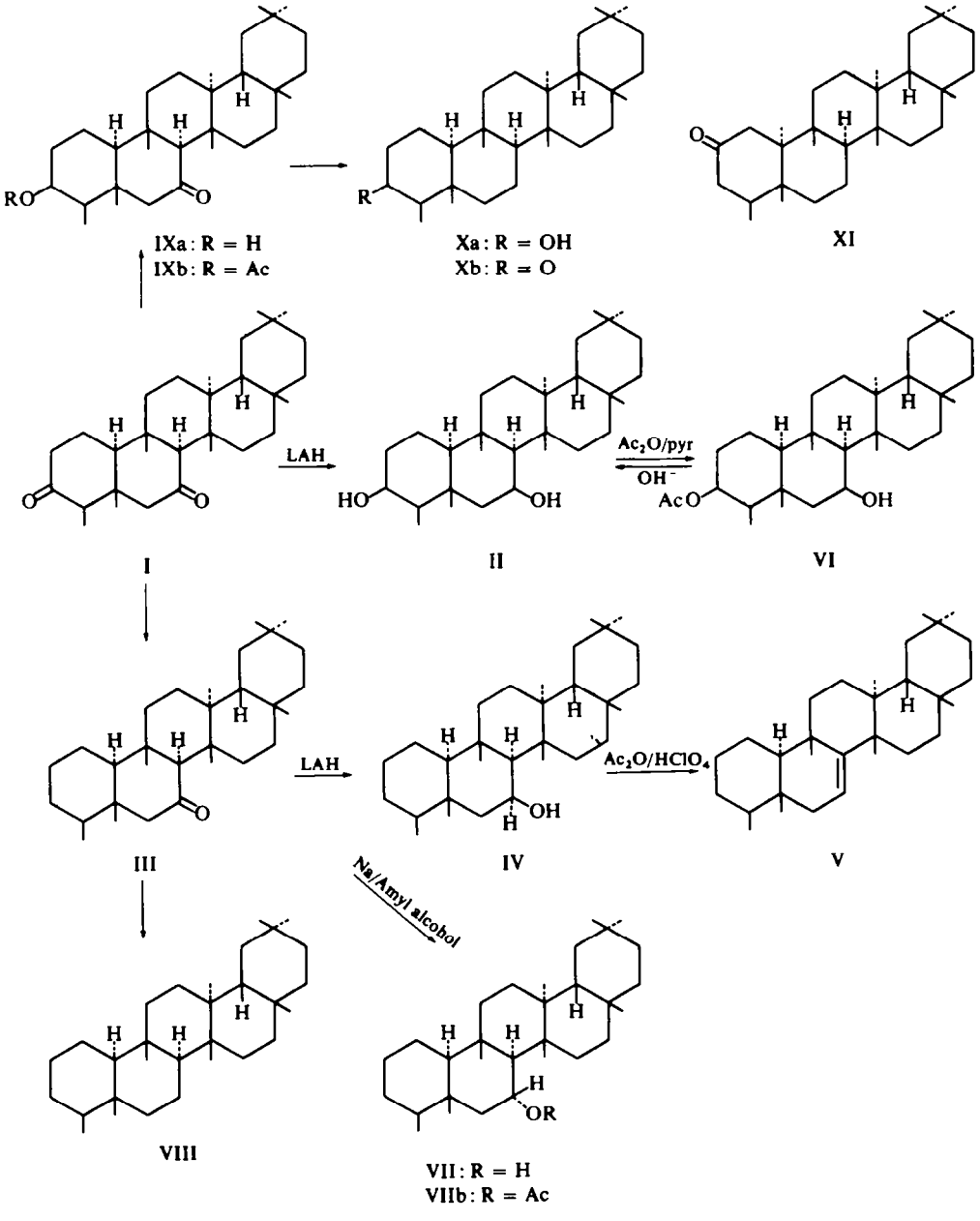
Abstract—The isolation of a new triterpene, putranjivadione, is described. By a combination of chemical and physical methods it was shown that putranjivadione is friedelane-3,7-dione (I).

Putranjiva roxburghii (Euphorbiaceae) is a common plant of tropical India the leaves and fruits[2] of which were used as indigenous medicine against colds and fever. We became interested in a chemical investigation of this plant as none has been reported in the literature.

The neutral material from the benzene extract of the whole plant yielded two crystalline compounds on chromatography over activated alumina. The less polar compound was shown to be identical with friedelin. The more polar constituent (m.p. 284–289°) having the molecular formula $C_{30}H_{48}O_2$ (mol. wt 440 by mass spectrometry) was named putranjivadione and has been shown to possess structure I. The two oxygen atoms present in the molecule were ketonic in nature (single absorption in the IR at 1710 cm^{-1}) and one of them proved to be highly hindered. A negative tetranitromethane test indicated that the compound lacked any ethylenic linkages and could thus tentatively be assigned to the friedelane or related group of pentacyclic triterpenes. The general appearance of the mass spectra of putranjivadione and its derivatives was consistent with the presence of a friedelane nucleus in these compounds[3] and the mass spectrometric fragmentation patterns will be discussed later in the paper.

The NMR spectrum of putranjivadione in $CDCl_3$ solution exhibited signals of the tertiary Me groups at 0·78, 0·92, 0·96, 1·00, 1·07, 1·18 and 1·42 ppm with the secondary Me doublet centered at about 0·88 ppm (J = 6 to 7 Hz). Other signals were not sufficiently separated to be identified, with the exception of a one-proton singlet at 2·87 ppm which is presumed to belong to the proton at C-8 which is α to the carbonyl and has no other proton neighbors. The same compound in benzene showed signals of tertiary Me groups at 0·59, 0·69, 1·02, 1·05, 1·08, 1·20 and 1·50 ppm with the secondary Me at 0·78 ppm and the one-proton signal at 2·58 ppm. The strong shift to higher field on going to benzene solvent for the Me groups at 0·78 and 0·92 ppm ($CDCl_3$) and the proton at C-8 (2·87 ppm) indicates[4] their proximity to one or both of the CO groups.

Putranjivadione on treatment with LAH gave a diol (IR absorption at 3610 cm^{-1}; mol. wt 444 by mass spectrometry) named putranjivadiol (II), treatment of which with acetic anhydride and pyridine yielded a monoacetate (VI) that displayed absorption in the IR at 3480 cm^{-1} (OH) and 1720 and 1265 cm^{-1} (acetate). The mol. wt of VI (486 by mass spectrometry) was in harmony with the presence of a single acetate function in this molecule and on alkaline hydrolysis the diol II was regenerated.



Huang Minlon reduction of putranjivadione (I) afforded a monoketone (III), $C_{30}H_{50}O$ (mol. wt 426 by mass spectrometry), which failed to give a hydrazone derivative while absorption at 1715 cm^{-1} in the IR indicated the presence of a keto function in a 6-membered ring.

The NMR spectrum in $CDCl_3$ solution of the monoketone III showed signals of the seven tertiary Me groups at 0·80, 0·87, 0·95, 0·99, 1·02, 1·18 and 1·40 ppm with the secondary Me at 0·73 ppm. The one-proton singlet observed in the diketone now appeared at 2·75 ppm. In addition, an AB pattern (2H), centered at 2·20 ppm due to the two isolated methylene protons at C-6 could now be observed, which was previously masked by the signals of protons α to the other CO group. In benzene the signals of the tertiary Me functions were observed at 0·73, 0·84, 0·97, 1·02, 1·07, 1·21 and 1·58 ppm with the secondary Me at 0·68 ppm. The one-proton singlet now appeared at 2·63 ppm and the AB pattern separated into a pair of doublets located at 2·26 and 1·84 ppm. The up-field shift of the proton singlet (2·75 ppm, $CDCl_3$) and one part of the AB pattern (2·17, $CDCl_3 \rightarrow$ 1·84 ppm, C_6H_6) identifies them as axial, as opposed to the equatorial one, which was shifted down-field (2·23 → 2·26 ppm).

Treatment of the monoketone under conditions suitable for exchange of protons α to a CO group with deuterium led to a monodeuterio derivative in which singlets remained at 2·75 and 2·23 ppm ($CDCl_3$), indicating that only the less hindered axial proton had exchanged.

The monoketone III on treatment with LAH furnished a mono-ol (IV) $C_{30}H_{52}O$ (mol. wt 428 by mass spectrometry), which had IR absorption at 3610 cm^{-1} and which failed to yield an acetate on treatment with acetic anhydride in pyridine solution. Such failure can be attributed to the severe hinderance of the axial OH group by the three axial Me groups at positions 5, 9 and 14.

The NMR spectrum of this material showed signals for the tertiary Me groups at 0·97, 0·98, 1·00, 1·08, 1·19, 1·24 and 1·42 ppm with the secondary Me doublet centered at 0·76 ppm. The proton (attached to C-7) adjacent to the OH group appeared at 4·45 ppm in a quartet-like pattern whose width was indicative of its having a total of two equatorial–axial and an equatorial–equatorial coupling interactions rather than an axial–equatorial and two axial–axial ones, thus confirming the axial nature of the OH group.

Treatment of the hydroxyfriedelane (IV) with acetic anhydride containing a drop of perchloric acid resulted in smooth dehydration to a hydrocarbon $C_{30}H_{50}$ (mol. wt 410 by mass spectrometry), which exhibited IR absorption at 815 cm^{-1} (tri-substituted double bond) consistent with structure V.

The NMR spectrum of V in $CDCl_3$ solution had signals for the tertiary Me groups at 0·83, 0·85 (two), 0·93, 0·97 and 1·08 ppm (two) with the secondary Me at 0·72 ppm. The olefinic proton appeared as a pair of doublets at 5·78 ppm ($J = 4$ and $J = 8$ Hz). In benzene these signals appeared at 0·84, 0·88, 0·96, 1·01, 1·03, 1·13 and 1·19 ppm with the secondary Me at 0·78 ppm, while the olefinic proton was located at about 5·85 ppm.

The monoketone III on reduction under epimerizing conditions with sodium and amyl alcohol yielded a second alcohol (VIIa), whose OH group must have the equatorial configuration since it could easily be acetylated in contrast to the axial epimer IV.

The hindered CO group of the monoketone III was resistant to reduction by the

procedure of Nagata and Itazaki.[5] However, using Barton's modification[6] of the Wolff–Kishner reduction, the ketone III was reduced in low yield to friedelane (VIII) (identified by a comparison of the mass and IR spectra with authentic material) thus confirming the basic skeleton of the molecule.

Putranjivadione (I) on reduction with sodium borohydride in methanol gave a hydroxy ketone (IXa), $C_{30}H_{50}O_2$ (mol. wt 442 by mass spectrometry), which on vigorous Wolff–Kishner reduction following Barton's conditions[6] yielded friedelanol (Xa; identical IR and mass spectra with an authentic sample and no mixture m.p. depression). Further characterization was effected by Jones' oxidation to friedelin (Xb; identical IR and mass spectra with authentic material). This series of reactions established the presence of the 3-keto-friedelane system in putranjivadione.

Mass spectra of putranjivadione and derivatives. The mass spectra of pentacyclic triterpenes containing the friedelane ring system have been described previously[3,7] and some of these correlations can be applied to the interpretation of the mass spectra of putranjivadione and derivatives.

Putranjivadione (I) and putranjivone (III) display mass spectra (Figs. 1 and 2) which contain a prominent peak at *m/e* 205. This ion has been attributed[3] to the species *a*, (*m/e* 205) being derived from rings D and E respectively and thus affords additional evidence for the absence of the oxygen functions in these rings in putranjivadione.

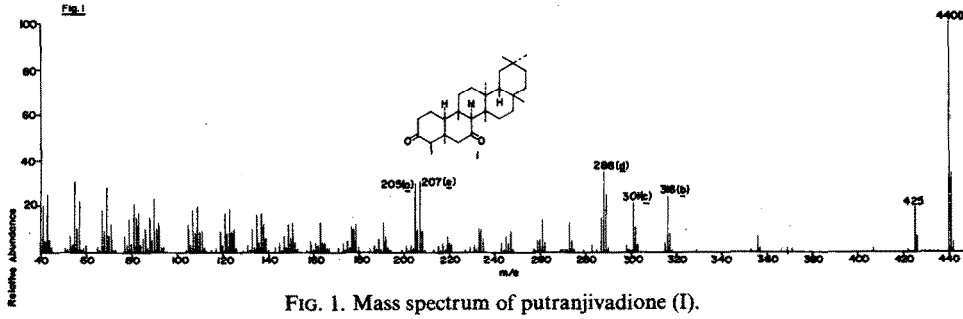


FIG. 1. Mass spectrum of putranjivadione (I).

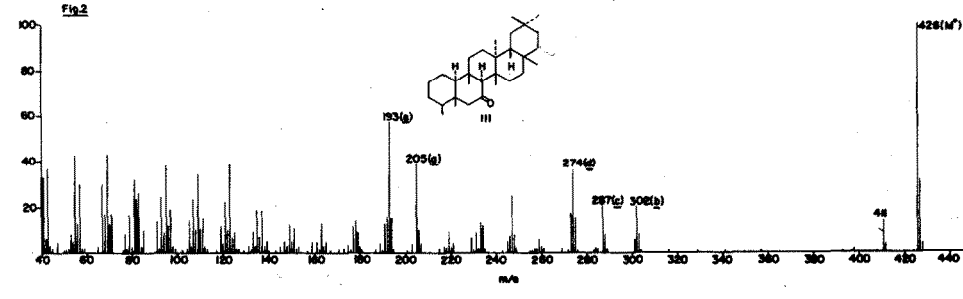
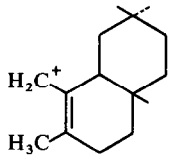


FIG. 2. Mass spectrum of putranjivone (III).

The remainder of the principal fragmentation modes of putranjivadione (I) appear to incorporate rings A and B (containing both keto functions) and portions of rings C and D respectively. Thus a peak at *m/e* 316 in the mass spectrum (Fig. 1) of putranjivadione (I) is located at *m/e* 302 in the spectra (Figs. 2, 3 and 4) of putranjivone (III),



a, *m/e* 205

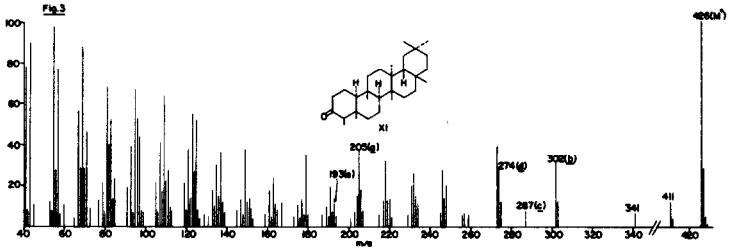


FIG. 3. Mass spectrum of friedelan-3-one (Xb).

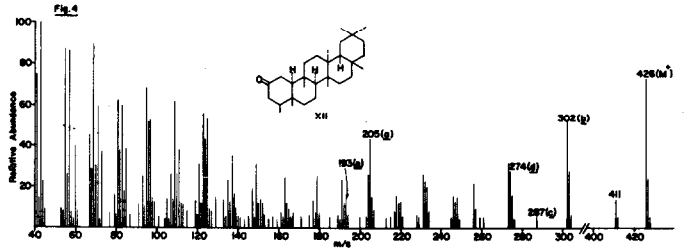


FIG. 4. Mass spectrum of friedelan-2-one (XI).

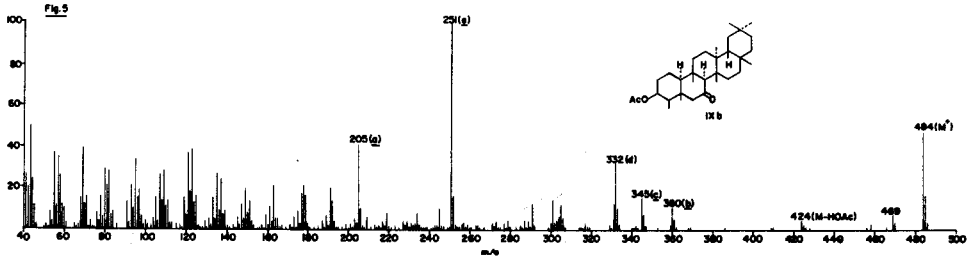
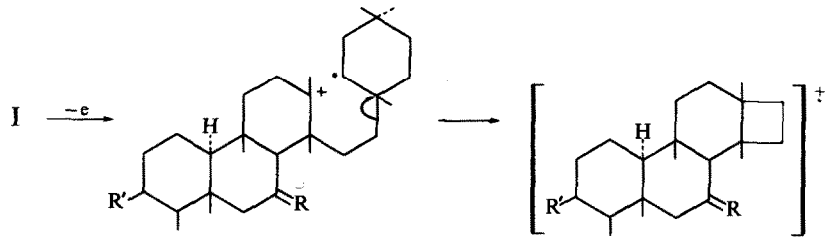


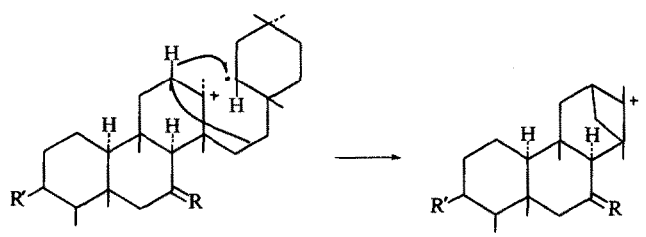
FIG. 5. Mass spectrum of 3-acetoxy-7-hydroxyfriedelane (IXb).

3-keto and 2-ketofriedelane (Xb and XI) respectively; a possible representation[3] for the responsible ion may be *b*. In harmony with this conclusion the mass spectrum (Fig. 5) of the acetoxy ketone (IXb) contains a peak at *m/e* 360.



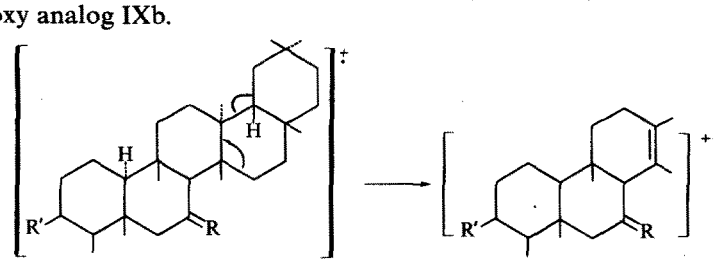
b R = R′ = O; *m/e* 316
R = H_2; R′ = O; *m/e* 302
R = O, R′ = OAc; *m/e* 360

A peak at *m/e* 301 in the mass spectrum (Fig. 1) of putranjivadione (I) is located at *m/e* 287 in the three monoketones III, Xb and XI and at *m/e* 345 in the acetoxyketone (IXb; Figs. 2, 3, 4 and 5 respectively). This ion may be visualized in terms of structure *c*, and one likely origin involving hydrogen transfer from C-12 is shown.



c { R = O, R′ = H_2; *m/e* 301
R = H_2, R′ = H_2; *m/e* 287
R = O, R′ = −OAc; *m/e* 345

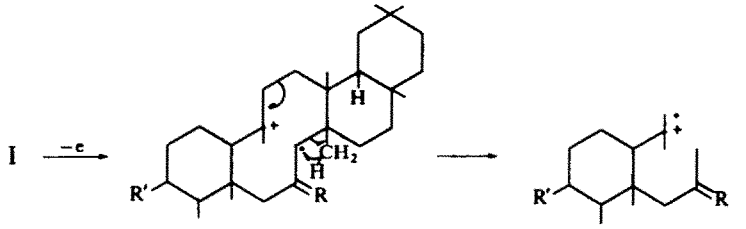
Another peak of diagnostic interest in the mass spectrum of putranjivadione (I) occurs at *m/e* 288. This could correspond to the species *d* and this assignment receives support from the location of a peak at *m/e* 274 in the spectra (Figs. 2–4) of putranjivone III, 3- and 2-ketofriedelane (Xb and XI) and at *m/e* 332 in that (Fig. 5) of the acetoxy analog IXb.



d { R = O, R′ = O; *m/e* 288
R = H_2, R′ = O; *m/e* 274
R = O, R′ = OAc; *m/e* 322

Finally, an ion of mass 207 in the mass spectrum (Fig. 1) of putranjivadione (I) may correspond to one of mass 193 in putranjivone (Fig. 2) and hence may be assigned structure *e*. It is noteworthy that the corresponding peak (*m/e* 193) in the

spectra of 2- and 3-ketofriedelane (Xb and XI) is of weak abundance. The base peak in the spectrum (Fig. 5) of the acetoxy ketone (IXb) occurs at *m/e* 251 and this can also be assinged structure *e*.



e { R = O, R' = O; *m/e* 207
R = H_2, R' = O; *m/e* 193
R = O, R' = OAc; *m/e* 251

The conclusion to be drawn from the mass spectra is that the second ketonic function must be located at C-6, C-7 or C-11; C-1 and C-2 are eliminated since putranjivadione is not an α or β diketone, and no aldehyde protons were visible in the NMR spectrum of putranjivadione (I). Since the NMR spectrum of III showed the presence of three hydrogens adjacent to the hindered CO group, only C-7 remains as the locus for the second ketonic function. The expression I for putranjivadione is thus established.

EXPERIMENTAL

All m.ps are uncorrected. The pet. ether used had b.p. 60–80°. NMR spectra were determined with Varian A-60 and HR-100 instruments. The mass spectra were determined with an Atlas CH-4 mass spectrometer using direct sample introduction into the ion source and 70 ev electrons. Sample identification was achieved by comparison of IR and where possible mass spectra. All derivatives (unless otherwise noted) yielded negative tetranitromethane tests for unsaturation. We wish to thank Mrs. Ruth Records of Stanford University for the ORD curves of putranjivadione (I) and putranjivone (III). All specific rotation values, unless stated otherwise, refer to $CHCl_3$ soln.

Extraction of Putranjiva roxburghii *wall.* Dried and powdered plant (1 kg) was extracted with benzene for 6 hr in a soxhlet apparatus. The neutral material (22 g) was chromatographed on activated alumina (400 g) and elution with pet. ether:benzene (2:3) afforded crude friedelin (0·1 g), m.p. 253–255°. Crystallization from $CHCl_3$–acetone yielded pure X, m.p. 257–261°, $[\alpha]_D$ −22·4°. (Found: C, 84·60; H, 11·91. $C_{30}H_{50}O$ requires: C, 84·44; H, 11·81%.)

Further elution of the chromatogram with pet. ether:benzene (1:4) furnished a solid (1 g), m.p. 270–275°, which on rechromatography over alumina and crystallization from $CHCl_3$–acetone afforded pure I, m.p. 284–289°, $[\alpha]_D$ −36·8°. (Found: C, 81·89; H, 10·97, mol. wt (mass spectrometry) 440. $C_{30}H_{48}O_2$ requires: C, 81·76; H, 10·98%; mol. wt 440.) ORD curve in dioxan (*c*, 0·08) $[\phi]_{589}$ −163°; $[\phi]_{319}^{trough}$ −5591°; $[\phi]_{312}$ −4206°; $[\phi]_{308}^{trough}$ −4792°, $[\phi]_{270}^{peak}$ +5058°.

LAH reduction of putranjivadione to putranjivadiol (II). Putranjivadione (500 mg) was added to a suspension of LAH (600 mg) in THF (80 ml), refluxed for 6 hr, allowed to stand overnight and worked up in the usual manner to yield crude diol (440 mg), m.p. 248–260°. Chromatography over activated alumina (50 g), elution with benzene:ether (3:2) and crystallization of the product from $CHCl_3$–MeOH yielded II, m.p. 270–274°, $[\alpha]_D$ +29·1°. (Found: C, 80·96; H, 11·50; mol. wt 444 (mass spectrometry). $C_{30}H_{52}O_2$ requires: C, 81·02; H, 11·79%; mol. wt 444.) The IR spectrum showed absorption at 3610 cm^{-1} (OH) and no CO absorption was evident.

Acetylation of putranjivadiol to putranjivadiol monoacetate (VI). Putranjivadiol (200 mg) in pyridine (2 ml) containing Ac_2O (2 ml) was warmed on the water-bath for 2 hr and then allowed to stand overnight at room temp. The product was crystallized from $CHCl_3$–acetone to furnish VI, m.p. 244–247°, $[\alpha]_D$ +38·0°. (Found: C, 79·25; H, 11·18. $C_{32}H_{54}O_3$ requires: C, 78·96; H, 11·18%.) IR absorption was noted

at 3480 cm^{-1} (OH) and 1720 and 1265 cm^{-1} (acetate). The monoacetate on hydrolysis with alcoholic KOH regenerated II.

Huang Minlon reduction of putranjivadione to putranjivone (III). Putranjivadione (640 mg) in diethylene glycol (53 ml) was refluxed with 85% hydrazine hydrate (6·5 ml) for 1 hr. KOH (640 mg) was added and the mixture was further refluxed for 1 hr when the condenser was removed and the mixture heated to 190°. After refluxing for an additional 2·5 hr the reaction mixture was cooled, diluted with water and the ppt filtered off. Chromatography on activated alumina (20 g) and elution with ether:benzene (4:1) yielded crystals (560 mg) which on crystallization from $CHCl_3$–MeOH yielded III, m.p. 260–263°, $[\alpha]_D$ +21·5°. (Found: C, 84·72; H, 11·73. $C_{30}H_{50}O$ requires: C, 84·44; H, 11·81%.) ORD curve in dioxan (*c*, 0·06); $[\phi]_{589}$ +95°; $[\phi]^{peak}_{524}$ +120°; $[\phi]^{shoulder}_{318-320}$ −149°; $[\phi]^{trough}_{312}$ −265°; $[\phi]^{shoulder}_{304}$ −60°; $[\phi]^{peak}_{266}$ +911°. IR absorption was recorded at 1715 cm^{-1} (6-membered ring ketone).

Putranjivone (100 mg) in pyridine (10 ml) containing 85% hydrazine hydrate (5 ml) and conc HCl (1 ml) was refluxed for 1 hr. On workup only putranjivone was recovered.

LAH reduction of putranjivone to putranjivol (IV). Putranjivone (500 mg) in THF soln was reduced with excess LAH to yield putranjivol (450 mg), m.p. 242–246°. Chromatography on alumina (20 g) and elution with pet. ether:benzene (7:3) followed by crystallization from $CHCl_3$–MeOH yielded IV, m.p. 245–248°, $[\alpha]_D$ +33·8°. (Found: C, 84·22; H, 12·14. $C_{30}H_{52}O$ requires: C, 84·04; H, 12·23%.) IR absorption was recorded at 3610 cm^{-1} (OH).

Putranjivol (200 mg) in pyridine (2 ml) containing Ac_2O (2 ml) was warmed on the water-bath for 5 hr and then allowed to stand overnight at room temp. Workup as usual yielded only putranjivol.

Dehydration of putranjivol with acetic anhydride and perchloric acid to putranjivene (V). Perchloric acid (1 drop) was added to a cooled suspension of putranjivol (200 mg) in Ac_2O (5 ml) and the mixture stirred at room temp during 20 min, poured into sat. $NaHCO_3$ aq and the ppt filtered off. Chromatography over alumina (10 g) and elution with pet. ether furnished a solid (180 mg) which on crystallization from $CHCl_3$–MeOH yielded V, m.p. 161–164°. $[\alpha]_D$ −58·7°. (Found: C, 87·58; H, 12·22. $C_{30}H_{50}$ requires: C, 87–73; H, 12·27%.) Putranjivene had IR absorption at 815 cm^{-1} (trisubstituted double bond) and the tetranitromethane test for unsaturation was positive.

Reduction of putranjivone with sodium and isoamyl alcohol to epi-putranjivol. Sodium (2 g) was slowly added to a refluxing soln of putranjivone (500 mg) in isoamyl alcohol (25 ml) and refluxing continued until all the Na had dissolved. After steam distillation the ppt was chromatographed over activated alumina (20 g). Elution with pet. ether:benzene (2:3) followed by crystallization from $CHCl_3$–MeOH yielded VIIa, m.p. 240–244°, $[\alpha]_D$ +16·2°. IR absorption at 3600 cm^{-1} (OH). (Found: C, 84·31; H, 12·19. $C_{30}H_{52}O$ requires: C, 84·04; H, 12·23%.)

Acetylation on heating for 3 hr at 100° with pyridine and Ac_2O followed by crystallization from $CHCl_3$–MeOH yielded the acetate VIIb with m.p. 216–218°. $[\alpha]_D$ ±0°. (Found: C, 81·93; H, 11·45. $C_{32}H_{54}O_2$ requires: C, 81·64; H, 11·56%.)

Wolff–Kishner reduction of putranjivone to friedelane. Sodium (500 mg) in diethylene glycol (20 ml) was heated to 180° (all temps measured with thermometer in liquid) and anhyd hydrazine (30 ml) added until the mixture refluxed at 180°. The soln was cooled, putranjivone (400 mg) added and the contents refluxed for 12 hr. A portion of the hydrazine was distilled from the reaction mixture until a reflux temp of 210° was attained. After refluxing for an additional 24 hr the reaction mixture was cooled and worked up in the usual manner. Chromatography over activated alumina (20 g) and elution with pet. ether furnished a solid (15 mg) which on crystallization from $CHCl_3$–MeOH yielded VIII, m.p. 245–247°, $[\alpha]_D$ +30·4°. The IR and mass spectra of this and authentic material were identical.

$NaBH_4$ *reduction of putranjivadione to the hydroxyketone* (IXa). Putranjivadione (800 mg) in pyridine (40 ml) was added to a soln of $NaBH_4$ (250 mg) in MeOH (20 ml) containing 1N NaOH (0·2 ml). After 100 hr the solution was acidified with 5N HCl (160 ml) and the product isolated with $CHCl_3$. Chromatography over activated alumina (40 g) and elution with pet. ether:benzene (1:4) yielded unreacted putranjivadione (150 mg). Further elution with benzene:ether (4:1) afforded colorless crystals (640 mg) which after crystallization from $CHCl_3$–MeOH yielded IXa, m.p. 303–306°, $[\alpha]_D$ +20·5°. (Found: C, 81·61; H, 11·25; mol. wt 442 (mass spectrometry). $C_{30}H_{50}O_2$ requires: C, 81·39; H, 11·38%; mol. wt 442.)

Acetylation with Ac_2O in pyridine soln and crystallization from $CHCl_3$–MeOH afforded IXb, m.p. 310–314°, $[\alpha]_D$ +36·0°. (Found: C, 79·59; H, 10·74; mol. wt 484 (mass spectrometry). $C_{32}H_{52}O_3$ requires: C, 79·28; H, 10·81%; mol. wt 484.)

Wolff–Kishner reduction of the hydroxy ketone (IXa) *to friedelanol* (Xa). Using an identical procedure as was used in the reduction of putranjivone (see above) IXa (500 mg) furnished a mixture which on

chromatography on alumina afforded a solid 15 mg. Crystallization from $CHCl_3$–MeOH yielded friedelanol m.p. 278–282° which had identical mass and IR spectra with authentic material. Jones' oxidation of this product gave Xb m.p. and mixture m.p. with authentic material m.p. 255–260° (identical IR and mass spectra with authentic material).

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