Tetrahedron Vol. 27, pp. 1545 to 1555 Pergamon Press 1971. Printed in Great Britain

BITTER PRINCIPLES OF *PICRASMA AILANTHOIDES* PLANCHON. NIGAKILACTONES A, B, C, D, E AND F[1]

T. MURAE, T. TSUYUKI, T. IKEDA, T. NISHIHAMA, S. MASUDA and T. TAKAHASHI

Department of Chemistry, Faculty of Science, The University of Tokyo, Bunkyo-ku, Tokyo, Japan

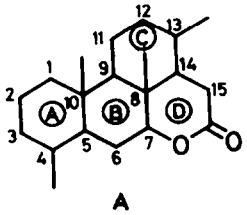
(*Received in Japan* 7 *November* 1970; *Received in the UK for publication* 18 *November* 1970)

Abstract—Five new crystalline bitter principles were isolated from the stem-chips of *Picrasma ailanthoides* Planchon (Simaroubaceae). Structures of nigakilactones A, B, C, E and F were shown to be II, III, IV, V and VI, respectively. A sixth constituent, nigakilactone D, was proved to be identical with quassin (I).

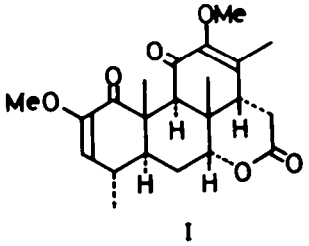
Picrasma ailanthoides Planchon (= *P. quassioides* Bennett) (Japanese name: nigaki, Simaroubaceae) is a shrub grown in Japan and China. Its stem and leaves taste considerably bitter and have been used as Chinese herb drugs. Shimoyama isolated from the plant a bitter principle, quassiin, $C_{31}H_{42}O_9$, which was poorly characterized.[2] Other earlier studies on the plant resulted in isolation of several compounds, none of which, however, was reported to be bitter to the taste.[3] In our first approach to the problem of isolating and purifying the constituents of the wood, we employed methods similar to those of Clark[4] and Robertson[5] to furnish no crystalline substance. The following method was eventually found to be successful.

A concentrated aqueous extract of the stem-chips was further extracted with benzene and the extract was passed through a neutral alumina column and separated into several crude fractions. Each fraction was further purified by repetition of silica gel dry column or TLC and of recrystallization giving rise to six crystalline bitter principles, which we have named nigakilactones A, B, C, D, E and F.

The bitter principles of various genera of the family Simaroubaceae have been investigated and their structures determined.[6] Spectral data of nigakilactones resemble one another and are closely related to those of quassin (I)[7] isolated from *Quassia amara* (Simaroubaceae) (Table 1). This provides convincing support for the presence in nigakilactones of the same skeletal structure (A) as that of quassin. Evidence establishing structures II, III, IV, V and VI for nigakilactones A, B, C, E and F, respectively, are reported in the present paper.[1] Nigakilactone D is shown to be identical with quassin (I).



A



I

TABLE 1. PMR SPECTRAL DATA (δ in ppm)[a]

Compounds	I	II	III	IV	V	VI	VII	VIII	IX	XI	XIII
t-CH_3	1·18 s 1·54 s	1·24 s 1·42 s	1·21 s 1·45 s	1·27 s 1·27 s	1·25 s 1·27 s 1·53 s	1·22 s 1·46 s 1·46 s	1·25 s 1·42 s	1·18 s 1·63 s	1·05 s 1·64 s	1·10 s 1·64 s	1·35 s 1·35 s 1·72 s
s-CH_3	1·11 d J = 7	1·01 d J = 6·5 1·10 d J = 6	1·00 d J = 6·5 1·13 d J = 6·5	1·01 d J = 6 1·06 d J = 7	1·08 d J = 7	1·11 d J = 7	0·89 d J = 6 1·11 d J = 6·5	1·06 d J = 6·5 1·12 d J = 7	1·10 d J = 6 1·17 d J = 6·5	1·05 d J = 6 1·13 d J = 6·5	1·13 d J = 7
C=C−CH_3	1·85 s	—	—	—	—	—	—	—	—	—	—
−O−CO−CH_3	—	—	—	1·95 s	1·98 s	—	2·06 s	2·17 s	—	—	—
−O−CH_3	3·54 s 3·65 s	3·54 s	3·60 s 3·65 s	3·42 s 3·54 s	3·55 s 3·57 s	3·58 s 3·73 s	3·51 s	3·56 s	3·55 s	3·45 s 3·53 s	3·61 s
C | C−C_7−O− | H	4·36 m	4·10 m	4·15 m	4·14 m	4·21 m	4·13 m	4·10 m	4·37 m	4·30 m	4·29 m	4·32 m
CH−OAc	—	—	—	5·22 q J = 11 J = 9	5·54 q J = 11 J = 9	—	4·80 q J = 11 J = 9	5·23 d J = 12	—	—	—
C=C−H	5·29 d J = 2	5·35 d J = 2·5	5·45 d J = 2·5	5·10 d J = 2·5	5·17 d J = 2·5	5·43 d J = 2	5·31 d J = 2	5·38 d J = 2	5·37 d J = 2·5	5·36 d J = 2·5	5·46 d J = 2·5

[a] Determined in $CDCl_3$ at 60 MHz. Coupling constants are expressed in Hz.
s: singlet, d: doublet, q: quartet, m: multiplet.

Nigakilactone A (II)

Nigakilactone A crystallized from aqueous methanol as colourless needles, m.p. 237·5–238° and $[\alpha]_D$ +53° (EtOH). Elemental analysis and the mass spectrum indicate the formula $C_{21}H_{30}O_6$ (M^+ at *m/e* 378). The IR spectrum in nujol shows OH absorptions at 3570 and 3490 cm^{-1}. The UV absorption at 272 nm (ε, 4800) and IR absorptions at 1680 and 1635 cm^{-1} indicate the presence of an α,β-unsaturated ketone function. An IR absorption at 1720 cm^{-1} is indicative of the presence of a lactone grouping in a 6-membered or larger ring. This received support from the PMR signal at δ 4·10 ppm (1H, m) due to proton at the lactone terminus (—CH—O—CO—). The PMR spectrum also indicates the presence of two secondary and two tertiary Me's, a OMe group and an olefinic proton (Table 1).

On acetylation with acetic anhydride in pyridine at room temperature, nigakilactone A gave a monoacetate (VII), which still shows an OH band in its IR spectrum. Oxidation of VII with sodium dichromate in acetic acid afforded a keto-acetate (VIII), whose IR spectrum shows no OH absorption. As four of the six O atoms were already characterized, the presence of two OH groups is shown for nigakilactone A. In the PMR spectrum of VII a quartet (1H, J = 11 and 9 Hz) due to proton on acetoxyl-bearing carbon (—CHOAc) appears at δ 4·80, and in the spectrum of VIII this quartet is changed to a doublet (J = 12 Hz) and shifted to down field (at δ 5·23). This indicates that the two OH groups are both secondary and in a relationship of α,β-diequatorial each other.

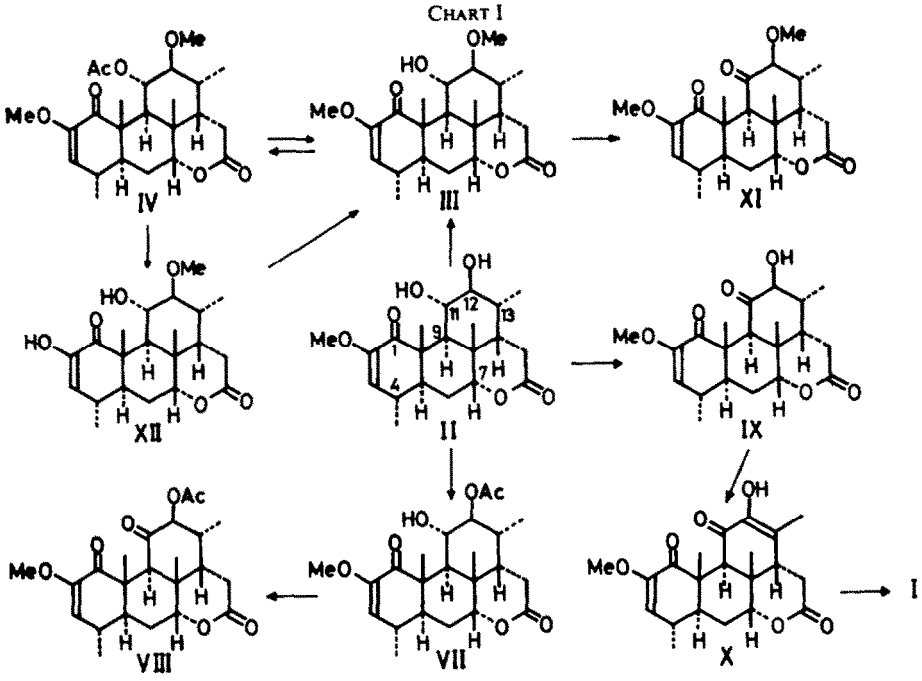
Oxidation of nigakilactone A with chromium trioxide in pyridine at room temperature gave an α-ketol (IX). Support for the α-ketol nature was obtained by the oxidation of IX to a diosphenol (X) with bismuth trioxide,[8] a reagent known to be able to transform α-ketols into diosphenols. The diosphenol (X) gave a deep blue colour with ferric chloride, and on addition of alkali its UV maximum in ethanol shifted from 270 nm to 313 nm. On methylation with dimethyl sulfate and alkali, X gave a methylated diosphenol, whose IR, UV, PMR, MS and $[\alpha]_D$ data are identical with those of quassin (I)[7] (Chart I). This confirms the presence of skeletal structure (A) in nigakilactone A.

These findings, along with the observation that the PMR spectrum of nigakilactone A (Table 1) shows the presence of one olefinic proton and the absence of vinyl methyl, lead to the location of α-glycol system on C-11 and C-12 (not on C-1 and C-2) for nigakilactone A. Thus the structure of nigakilactone A is established as II, except the stereochemistry of ring C. As described below, the stereostructure of nigakilactone A is as in II by correlation between nigakilactone A and nigakilactone C.

Nigakilactones B (III) *and C* (IV)

The molecular formula of nigakilactone B, $C_{22}H_{32}O_6$, m.p. 278·5°, $[\alpha]_D$ +17° (EtOH), was determined by elemental analysis and the appearance of the M^+ peak at *m/e* 392 in the mass spectrum. The IR (3460, 1675 and 1630 cm^{-1}) and UV (λ_{max} 272 nm; ε, 6700) spectra reveal characteristic absorptions for OH group and an α,β-unsaturated ketone. The PMR signal at δ 4·15 (1H, multiplet) and an IR absorption at 1725 cm^{-1} are indicative of the presence of a lactone grouping in a 6-membered or larger ring. Furthermore, the PMR spectrum shows the presence of two secondary and two tertiary Me's, two OMe groups and an olefinic proton (Table 1). Oxidation of nigakilactone B with sodium dichromate in acetic acid gave a ketone (XI),

CHART I



whose IR spectrum showed no OH band. As five of the six O atoms were already characterized, the presence of only one OH group is shown for nigakilactone B. In the PMR spectrum of XI a doublet (1H, J = 12 Hz) due to proton geminal to OMe and α to CO group (O=C—CHOMe) appears at δ 3·60. This suggests the presence in nigakilactone B of an OH adjacent to a OMe group.

Nigakilactone C, m.p. 252·5–253°, $[\alpha]_D$ +9° (EtOH) has the composition $C_{24}H_{34}O_7$ (M^+ at m/e 434). The UV spectrum (λ_{max} 265 nm; ε, 4300) shows characteristic band for an α,β-unsaturated ketone. The PMR signal at δ 4·14 (1H, m) and an IR absorption at 1730 cm^{-1} are indicative of the presence of a lactone grouping in a 6-membered or larger ring. The PMR (δ 1·95, 3H, s), IR (1735 and 1240 cm^{-1}) and MS [m/e 402, $(M\text{-AcOH})^+$] spectra show the presence of an acetoxyl group. In the PMR spectrum there appear signals due to two secondary and two tertiary Me's, two OMe groups and an olefinic proton (Table 1).

PMDR experiments (in C_6D_6, at 100 MHz) on nigakilactone C afforded evidence for the presence of partial structure (B) (Fig 1). Irradiation on the proton quartet at δ 5·37 (H_b) causes a collapse of the other proton quartet at δ 2·90 (H_c) into a doublet and changes the proton doublet at δ 2·66 (H_a) into a singlet. The coupling constants of H_a—H_b (J = 11 Hz), H_b—H_c (J = 9 Hz) and H_c—H_d (J = 11 Hz) indicate that the four adjacent protons are in axial–axial relationships.

The presence of methylated diosphenol moiety was shown for nigakilactones B and C. Treatment of nigakilactone C with hydrochloric acid in acetic acid afforded nornigakilactone B (XII), which gave a deep blue colour with ferric chloride. On addition of alkali the UV maximum of XII in ethanol shifted from 280 nm to 335 nm. Nornigakilactone B (XII) was easily methylated with diazomethane to give nigakilactone B. Thus, the α,β-unsaturated carbonyl grouping for nigakilactones B and C

can now be expanded to methylated diosphenol moiety, as in the case of nigakilactone A (II).

Nigakilactones A, B and C were shown to be closely related lactones in the following way. Nigakilactone B was obtained by methylation of nigakilactone A with methyl iodide, silver oxide and dimethylformamide. Acetylation of nigakilactone B with acetic anhydride in pyridine gave nigakilactone C, which on alkaline hydrolysis

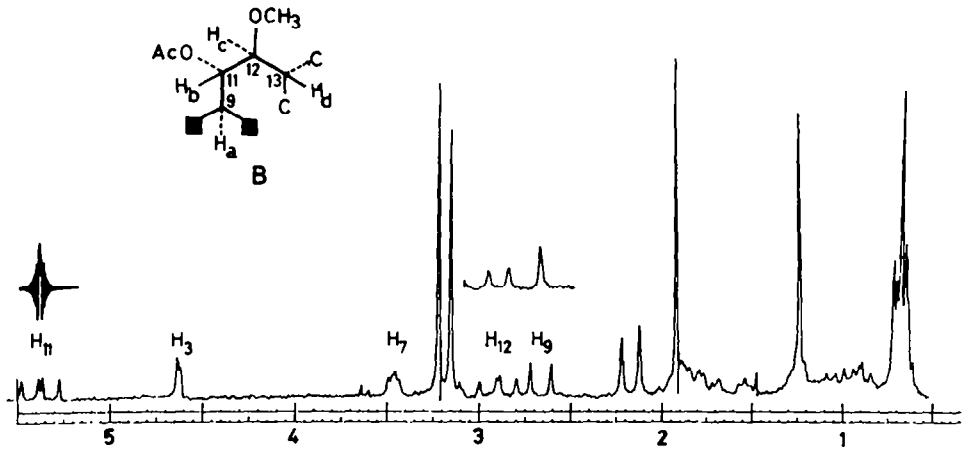


FIG 1. PMR and PMDR spectra of nigakilactone C (IV) in C_6D_6 at 100 MHz. (■ indicates carbon with no proton)

regenerated nigakilactone B (Chart I). This shows that nigakilactone C is a monoacetate of nigakilactone B, which in turn is a monomethyl ether of nigakilactone A. As conversion of nigakilactone A into quassin (I) was already realized, the presence of skeletal structure (A) is shown for nigakilactones B and C.

These observations, along with PMR and PMDR data given above, lead to the location of partial structure (B) on ring C and of methylated diosphenol moiety on ring A for nigakilactone C. The stereostructures III and IV are thus established for nigakilactones B and C, respectively. The stereochemistries at C-11, C-12 and C-13 of nigakilactone A must be the same as those of nigakilactones B and C. Therefore the stereostructure of nigakilactone A should be represented by II.

The UV maximum at 271–273 nm of nigakilactone A (II), nigakilactone B (III) and of VII shows the presence of H-bonding between the OH at C-11 and the CO group at C-1. This provides support for the location of the OH group on C-11 for these nigakilactones. The absorption maximum at 263–265 nm is observed for nigakilactone C (IV) and 11-keto-compounds (VIII, IX and XI), which are lacking such an OH group.

Nigakilactones E (V) *and F* (VI)

The analysis of nigakilactone E, m.p. 280°, $[\alpha]_D$ +36° (EtOH), fitted best for molecular formula $C_{24}H_{34}O_8$ (M^+ at *m/e* 450). The IR (3460, 1740, 1725 sh, 1717, 1642 and 1255 cm^{-1}) and UV (λ_{max} 264 nm; ε, 4600) spectra, along with the PMR (δ 4·21, 1H, m; δ 1·98, 3H, s) and MS spectra [*m/e* 390, $(M\text{-AcOH})^+$], suggest the presence of a lactone grouping in a 6-membered or larger ring, an α,β-unsaturated CO system and an

acetoxyl group, together with that of the OH group. The PMR spectrum (Table 1) shows the presence of one secondary and three tertiary Me's, two OMe groups and an olefinic proton. Of the eight O atoms, seven are involved in the above functional groups other than OH. Therefore one OH group should be present in nigakilactone E.

The molecular formula of nigakilactone F, $C_{22}H_{32}O_7$, m.p. 265–265·5°, $[\alpha]_D$ +46° (EtOH), was determined by elemental analysis and the mass spectrum (M^+, at *m/e* 408). The UV spectrum (λ_{max} 272 nm; ε, 4500) shows characteristic absorption for an α,β-unsaturated CO. An IR absorption at 1732 cm^{-1} and PMR signal at δ 4·13 (1H, m) suggest the presence of a lactone grouping in a 6-membered or larger ring. The IR spectrum shows also OH absorptions at 3530, 3470 (sh) and 3450 cm^{-1}. In the PMR spectrum there appear signals due to one secondary and three tertiary Me's, two OMe groups and one olefinic proton. Nigakilactone F was obtained by alkaline hydrolysis of nigakilactone E. Thus, nigakilactone E is a monoacetate of nigakilactone F, and two OH groups must be present in nigakilactone F.

The PMR spectra (Table 1) of nigakilactones E and F are best interpreted on the basis of the skeletal structures of nigakilactones C (IV) and B (III), respectively. The marked differences between the two pairs of compounds are that nigakilactones E and F contain one secondary and three tertiary Me's, while nigakilactones C and B two secondary and two tertiary Me's. Furthermore, nigakilactones E and F contain one OH group more than nigakilactones C and B, respectively. The extra OH group can only be placed at either C-13 or C-4, if the presence of skeletal structure (A) is assumed for nigakilactones E and F. The splitting pattern of the olefinic proton signal around δ 5·1–5·5 of nigakilactones E and F is the same as that of C-3 olefin proton signal of nigakilactones A, B and C. Therefore the OH group is suggested to be located on C-13 (not on C-4).

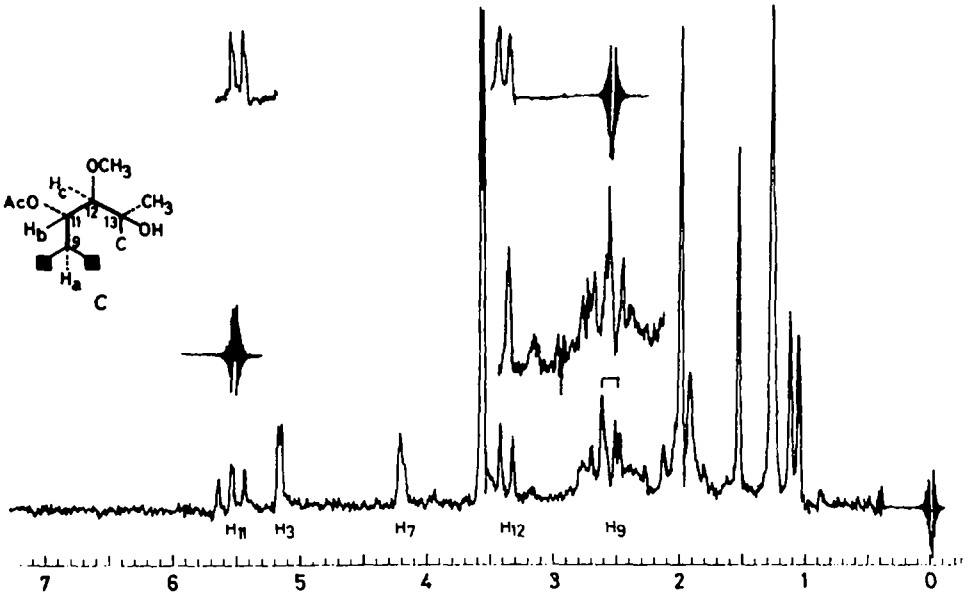
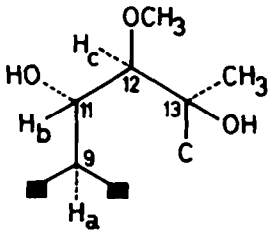


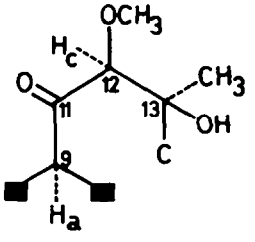
FIG 2. PMR and PMDR spectra of nigakilactone E (V) in $CDCl_3$ at 100 MHz.

PMDR experiments (in $CDCl_3$, at 100 MHz) of nigakilactone E afforded evidence for the presence of partial structure (C) (Fig 2). Irradiation on the proton quartet at δ 5·54 (H_b) causes a collapse of the proton doublet at δ 3·38 (H_c) into a singlet, and the other proton doublet at δ 2·57 (H_a) into a singlet. In the reverse experiment, irradiation on the doublet at δ 2·57 (H_a) changes the quartet at δ 5·54 (H_b) into a doublet. The coupling constants of H_a—H_b (J = 11 Hz) and H_b—H_c (J = 9 Hz) show that the three adjacent protons are in axial–axial relationships.

Oxidation of nigakilactone F with sodium dichromate in acetic acid gave a ketone (XIII). In the PMR spectrum of nigakilactone F two doublet signals appear at δ 3·03 (H_c, J = 9 Hz) and δ 2·48 (H_a, J = 11 Hz) (partial structure D), and in the spectrum of XIII both of the signals are changed to a singlet and shifted to down field [at δ 3·88 (H_c) and δ 2·66 (H_a) respectively] (partial structure E). The proton quartet at δ 4·00 (H_b; J = 11 and 9 Hz) observed in the spectrum of nigakilactone F disappears in that of XIII. These spectral data are compatible with those of nigakilactone E.



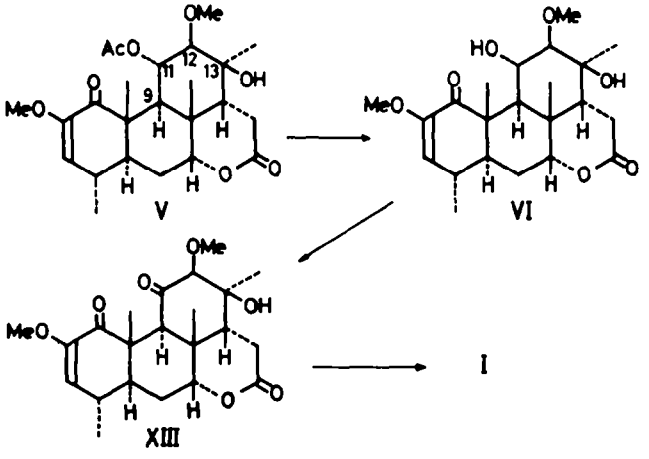
D



E

On refluxing with sodium acetate in acetic anhydride, XIII afforded a dehydrated product, whose IR, UV, PMR, MS and $[\alpha]_D$ data were identical with those of quassin (I)[7] (Chart II). The presence in nigakilactone F (and in nigakilactone E) of skeletal structure (A) is thus confirmed. The facile dehydration of the ketone (XIII) to yield I suggests that the OH group at C-13 is in axial conformation.

CHART II



Partial structures (C and D) can only be placed at ring C for nigakilactones E and F, respectively. The methylated diosphenol moiety must be located on ring A. Therefore the stereostructure of nigakilactone E is determined to be V. The stereostructure VI follows for nigakilactone F.

The UV maximum at 272 nm, showing the presence of hydrogen bonding between the OH group at C-11 and the CO at C-1, provides support for the location of the OH group on C-11 for nigakilactone F (VI). Nigakilactone E (V) and the ketone (XIII), which are lacking such an OH group, show an absorption maximum at shorter wave length (264 nm).

Nigakilactone D (I)

The molecular formula of $C_{22}H_{28}O_6$ (M^+ at *m/e* 388) was given for nigakilactone D, m.p. 219–220°, $[\alpha]_D$ +23° (EtOH), which was shown to be identical with quassin (I)[7] in all respects (m.p., IR, UV, PMR, MS, TLC and $[\alpha]_D$).

Finally, the presence of two other new bitter principles, picrasin B[9a] and picrasin A,[9b] in the same plant has recently been reported by Hikino *et al.*, and their structures have been shown to be a quassin derivative whose ring A is saturated[9a] and a compound related to simarolide,[9b] respectively.

EXPERIMENTAL

IR, UV and Mass spectra were measured using Hitachi EPI-G2, Hitachi EPS-3 and Hitachi RMU-6 spectrometers, respectively. PMR spectra were taken on a JEOL JNM-C-60 spectrometer at 60 MHz in $CDCl_3$ soln containing TMS as an internal standard, unless otherwise stated. Chemical shifts are expressed in δ (ppm downfield from TMS). The PMDR experiments were made using a Varian HA-100 spectrometer at 100 MHz in C_6D_6 soln for nigakilactone C (IV) (Fig 1), and using a JEOL 4H-100 spectrometer at 100 MHz in $CDCl_3$ soln for nigakilactone E (V) (Fig 2). All m.ps were determined on a hot block and are reported uncorrected.

Isolation. The stem-chips (160 Kg) of *Picrasma ailanthoides* Planchon were ground into powder and extracted three times with boiling water. The aqueous extract was concentrated under reduced pressure and extracted with benzene. Evaporation of the solvent gave a dark brown residue (70 g) which tasted very bitter. The residue was chromatographed on alumina (2 Kg, Showa Chemical Co.; treated with dil HCl, washed with water and dried at 110° for 6 hr). The eluted fractions (each 3 l) were collected and examined by TLC (silica gel).

Fractions 14–18 (eluent:ether) were combined and the solvent was distilled off. The residue (2·3 g) was further chromatographed on silica gel dry column (250 g, Wako-gel C-200). Each fraction eluted with AcOEt-ether (1:2) (each 50 ml) was tested for TLC (silica gel). The fractions 10–16 thus obtained was combined and the solvent distilled off. The residue was crystallized from benzene-light petroleum to give *nigakilactone C* (IV) (0·7 g) as colourless needles, m.p. 252·5–253°; $[\alpha]_D$ +9° (*c* 0·28, in EtOH); UV (MeOH) λ_{max} 265 nm (ε 4300); IR (Nujol) ν_{max} 1735, 1730, 1700, 1625, 1236 cm^{-1}. (Found: C, 66·25; H, 8·25. Mol. wt. by mass spectrum 434. Calc. for $C_{24}H_{34}O_7$: C, 66·34; H, 7·89%. Mol. wt. 434). PMR data are registered in Table 1.

Fractions 19–21 (eluent:ether) gave a residue (1 g), which was chromatographed on silica gel dry column (150 g) (eluent: AcOEt-ether, 1:1; each fraction 50 ml). The fractions 8–13 gave *nigakilactone B* (III) which crystallized from benzene-light petroleum (0·3 g) as colourless needles, m.p. 278·5°; $[\alpha]_D$ +17° (*c* 0·19, in EtOH); UV (EtOH) λ_{max} 272 nm (ε 6700); IR (Nujol) ν_{max} 3460, 1725, 1675, 1630 cm^{-1}; PMR (Table 1). (Found: C, 67·28; H, 7·99. Mol. wt. by mass spectrum 392. Calc. for $C_{22}H_{32}O_6$: C, 67·32; H, 8·22%. Mol. wt. 392).

Fractions 27–34 (eluent: AcOEt-ether 1:1) gave a residue (4·9 g) which was chromatographed on silica gel dry column (400 g) (eluent: AcOEt-ether, 1:1; each fraction 150 ml) to give the fractions 7–9 containing I and V. These fractions were combined and evaporation of the solvent gave a residue (4·1 g). The residue was further chromatographed on silica gel dry column (200 g) (eluent: AcOEt-ether, 1:1; each fraction 50 ml).

The fractions 5–7 gave a solid which was crystallized from aqueous MeOH (1 g) to give *nigakilactone D* (I) as colourless needles, m.p. 219–220°; $[\alpha]_D$ +23° (*c* 0·26, in EtOH); UV (EtOH) λ_{max} 255 nm (ε 12,600); IR (Nujol) ν_{max} 1742, 1700, 1680, 1626, 1632 cm^{-1}; PMR (Table 1). (Found: C, 67·81; H, 7·55. Mol. wt. by mass spectrum 388. $C_{22}H_{28}O_6$ requires: C, 68·02; H, 7·27%. Mol. wt. 388). This substance was shown to be identical with the authentic specimen of I in all respects. The fractions 8–12 gave a residue which was chromatographed on silica gel dry column (eluent: AcOEt-benzene, 1:1). Fractional crystallization from acetone followed by crystallization from benzene-light petroleum afforded *nigakilactone E* (V) as colourless needles (0·3 g), m.p. 280°, $[\alpha]_D$ +36° (*c* 0·22, in EtOH); UV (MeOH) λ_{max} 264 nm (ε 4600); IR (Nujol) ν_{max} 3460, 1740, 1725 sh, 1717, 1642, 1255 cm^{-1}; PMR (Table 1). (Found: C, 63·93; H, 8·22. Mol. wt. by mass spectrum 450. Calc. for $C_{24}H_{34}O_8$: C, 63·98; H, 7·61%. Mol. wt. 450).

Fractions 35–38 (eluent: AcOEt-ether, 1:1) gave a residue which was chromatographed on silica gel dry column (eluent: AcOEt-ether, 1:1) followed by crystallization from aqueous MeOH to afford *nigakilactone F* (VI) (0·5 g) as colourless needles, m.p. 265-265·5°; $[\alpha]_D$ +46° (*c* 0·20, in EtOH); UV (MeOH) λ_{max} 272 nm (ε 4500); IR (Nujol) ν_{max} 3530, 3470 sh, 3450, 1732, 1684, 1675, 1642, 1634 cm^{-1}; PMR (Table 1). (Found: C, 64·77; H, 8·05. Mol. wt. by mass spectrum 408. $C_{22}H_{32}O_7$ requires: C, 64·68; H, 7·90%. Mol. wt. 408).

Fractions 42–47 (eluent: AcOEt) gave a residue (3·4 g) which was chromatographed on silica gel dry column (eluent: AcOEt-benzene, 2:1) followed by crystallization from aqueous MeOH to give *nigakilactone A* (II) as colourless needles (1 g), m.p. 237·5–238°; $[\alpha]_D$ +35° (*c* 0·26, in EtOH); UV (EtOH) λ_{max} 271 nm (ε 4800); IR (Nujol) ν_{max} 3570, 3490, 1720, 1680, 1635 cm^{-1}; PMR (Table 1). (Found: C, 63·97; H, 8·10. Mol. wt. by mass spectrum 378. Calc. for $C_{21}H_{30}O_6\,H_2O$: C, 63·61; H, 8·14%. Mol. wt. 378).

Quassin. Authentic sample of I was prepared as follows. Crude quassin supplied by Koch-Light Laboratories Ltd. was found to consist of a mixture of quassin and neoquassin. The crude quassin was dissolved in aqueous EtOH and treated with freshly prepared silver oxide for 15 hr under reflux. The warm mixture was filtered and the filtrate was diluted with water and extracted with $CHCl_3$. The solvent was distilled off and the residue was purified by silica gel chromatography and by recrystallization from benzene-light petroleum to give colourless crystals whose m.p., IR, UV and PMR spectra were identical with those of the literature.[7]

Acetylation of nigakilactone A. Nigakilactone A (II) (42 mg) dissolved in pyridine (1 ml) was treated with Ac_2O (1 ml) for 1·5 hr at room temp. Crystallization from benzene-light petroleum gave VII as colourless needles (30 mg), m.p. 224–225°; UV (MeOH) λ_{max} 273 nm (ε 4000); IR (Nujol) ν_{max} 3430, 1740, 1720, 1685, 1630 cm^{-1}; PMR (Table 1); Mass spectrum, *m/e* 420 (M^+), 360 (M-AcOH)$^+$.

Keto-acetate (VIII). A soln of $Na_2Cr_2O_7$ (200 mg) in AcOH (2 ml) was added to a soln of VII (40 mg) in AcOH (1 ml), and the mixture was kept overnight at room temp, diluted with water, neutralized with Na_2CO_3, and extracted with $CHCl_3$. Evaporation of the solvent gave a residue which, on crystallization from benzene-light petroleum, afforded VIII as colourless needles, m.p. 230–231·5°; UV (EtOH) λ_{max} 264 nm (ε 5000); IR (Nujol) ν_{max} 1740, 1730, 1700, 1635 cm^{-1}, absence of ν_{O-H}; PMR (Table 1).

α-Ketol (IX). Chromium trioxide (100 mg) in pyridine (1·5 ml) was added to a soln of II (109 mg) in pyridine (3 ml), and kept at room temp for 28 hr. Water (50 ml) was added and extracted with $CHCl_3$. Evaporation of the solvent gave a residue, which was separated by preparative TLC to give II (27 mg) and IX (21 mg), IR (Nujol) ν_{max} 3450, 1720, 1685, 1630 cm^{-1}; UV (EtOH) λ_{max} 263 nm; PMR (Table 1).

Diosphenol (X). Bismuth trioxide (35 mg) was added into a soln of IX (21 mg) in AcOH (10 ml) and kept for 20 hr at 100°. After evaporation of the solvent *in vacuo* $CHCl_3$ was added and insoluble materials were filtered off. The filtrate was evaporated to furnish X as amorphous solids (19 mg), UV (MeOH) 270 nm (ε 11,200), shifted to 313 and 263 nm in alcoholic alkali; IR (Nujol) 3420, 1730, 1700, 1660, 1630 cm^{-1}. The diosphenol (X) gave a deep blue colour with ferric chloride.

Methylation of the diosphenol (X). Me_2SO_4 (1·5 ml) was added into a soln of X (19 mg) in 2N NaOH (4 ml), and the mixture was stirred for 1 hr at room temp, neutralized with 2N NaOH and extracted with $CHCl_3$. The extract was passed through an alumina column and eluted with $CHCl_3$. Earlier fractions consisted of Me_2SO_4 and evaporation of the solvent of later fractions followed by crystallization from benzene-light petroleum gave colourless needles (14 mg), which were shown to be identical with I by IR, UV, PMR, MS, TLC, $[\alpha]_D$ and mixed m.p.

Oxidation of nigakilactone B (III). To a soln of III (130 mg) in AcOH (3 ml), $Na_2Cr_2O_7$ (200 mg) was added and the mixture was kept for 4 hr at room temp, neutralized with $NaHCO_3$ and extracted with $CHCl_3$. Evaporation of the solvent gave a residue which was purified by preparative TLC followed by crystallization from benzene-light petroleum to afford XI as colourless needles, m.p. 258°; UV (EtOH)

λ_{max} 263 nm (ε 5000); IR (Nujol) ν_{max} 1730, 1700, 1635 cm^{-1}, absence of ν_{O-H}; PMR (Table 1); Mass spectrum, *m/e* 390 (M^+) ($C_{22}H_{30}O_6$).

Demethylation of nigakilactone C (IV). A mixture of IV (46 mg), 2N HCl (6 mg) and AcOH (2 mg) was heated on steam bath for 1·5 hr, cooled, alkalined with KOH aq, saturated with CO_2 and kept for 12 hr at room temp. Extraction with $CHCl_3$ and evaporation of the solvent gave XII (40 mg), UV (EtOH) λ_{max} 219, 280 nm, shifted to 217, 335 nm in alcoholic alkali; IR (Nujol) ν_{max} 3430, 1720, 1680, 1660, 1635 cm^{-1}. The compound XII gave III by methylation with diazomethane.

Methylation of nigakilactone A (II). A sealed tube containing II (23 mg), MeI (6 ml), Ag_2O (57 mg) and DMF (3 ml) was heated at 80° for 4 hr. DMF and MeI were evaporated *in vacuo* and the residue was chromatographed on silica gel dry column (60 g) (eluent: ether; each fraction 150 ml). The fractions 15–17 gave a residue which was crystallized from benzene-light petroleum to afford colourless crystals (2·5 mg), whose identity with III was shown by TLC, IR, UV and $[\alpha]_D$.

Acetylation of nigakilactone B (III). A mixture of III (24 mg), Ac_2O (3 ml) and pyridine (5 ml) was heated on steam bath for 12 hr, and after addition of MeOH the solvent was evaporated *in vacuo*. The acetylated product was purified by preparative TLC and by crystallization from benzene-light petroleum to afford colourless needles (6 mg), which was identical with IV.

Hydrolysis of nigakilactone C (IV). Nigakilactone C (IV) in 2% ethanolic KOH was heated under reflux for 2 hr. The solvent was evaporated and the residue was dissolved in water. After saturation with CO_2, the aqueous soln was set aside overnight at room temp. Extraction with $CHCl_3$ and evaporation of the solvent gave a residue. Recrystallization from benzene-light petroleum afforded colourless needles, which was shown to be identical with III.

Hydrolysis of nigakilactone E (V). Nigakilactone E (V) was refluxed in 1% ethanolic KOH for 2 hr. The solvent was distilled off and the residue was dissolved in water (15 ml). After saturation with CO_2, the aqueous soln was kept overnight at room temp, then extracted with $CHCl_3$. The extract was purified by preparative silica gel TLC followed by crystallization from benzene-light petroleum ether to afford VI.

Oxidation of nigakilactone F (VI). A soln of $Na_2Cr_2O_7$ (900 mg) in AcOH (9 ml) was added to VI (153 mg) in AcOH (10 ml), and the mixture was set aside for 5·5 hr at room temp, diluted with water, neutralized with $NaHCO_3$, and extracted with $CHCl_3$. Evaporation of the solvent gave a residue which was crystallized from benzene to afford XIII as colourless needles (103 mg), m.p. 256·5–257°; UV (MeOH) λ_{max} 264 nm (ε 5700); IR (Nujol) ν_{max} 3550, 3480, 1730, 1696, 1630 cm^{-1}; PMR (Table 1); Mol. wt. by mass spectrum 406.

Dehydration of the ketone (XIII). A mixture of XIII (67 mg), NaOAc (300 mg) and Ac_2O (3 ml) was heated under reflux for 2 hr, and after cooling EtOH was added to the mixture. Evaporation of AcOEt and excess of EtOH gave a residue, which was dissolved in $CHCl_3$. Insoluble materials was filtered off and the filtrate was evaporated to dryness. Crystallization of the residue from benzene-light petroleum afforded I (32 mg), whose identity with the authentic specimen was confirmed by TLC, IR, UV, PMR, MS and $[\alpha]_D$.

Acknowledgements—The authors wish to thank Professor T. Kubota, Osaka City University, and Professor O. Simamura, the University of Tokyo, for suggestion to initiate this work and for encouragement. We are grateful to Dr. I. Chuman, Teikoku Hormone Manufacturing Co., Ltd., for the extraction, and to Dr. T. Nishida, Nippon Electric Varian, Ltd., for PMDR experiments.

REFERENCES

[1] Preliminary communications:
[a] T. Murae, T. Tsuyuki, T. Nishihama, S. Masuda and T. Takahashi, *Tetrahedron Letters* 3013 (1969);
[b] T. Murae, T. Ikeda, T. Tsuyuki, T. Nishihama and T. Takahashi, *Bull. Chem. Soc. Japan* **43**, 969 (1970)

[2] J. Shimoyama and K. Hirano, *Apotheker Z.* **7**, 459 (1892)

[3] I. Miura, *J. Chem. Soc. Japan* (Ind. Chem. Sec.), **26**, 568 (1923); Y. Kimura, *Ann. Repts. Metrop. Instit. Hygien. Sci. Tokyo* **4**, 116 (1953); N. Inamoto, S. Masuda, O. Simamura and T. Tsuyuki, *Bull. Chem. Soc. Japan* **34**, 888 (1961); Y. Kimura, M. Takido and S. Koizumi, *J. Pharm. Soc. Japan* (Yakugaku Zasshi), **87**, 1731 (1967)

[4] E. P. Clark, *J. Am. Chem. Soc.* **59**, 927, 2511 (1937)

[5] E. London, A. Robertson and H. Worthington, *J. Chem. Soc.* 3431 (1950); K. R. Hanson, D. B. Jaquiss, J. A. Lamberton, A. Robertson and W. E. Savige, *Ibid.* 4238 (1954)

[6] Cf. *E.g.*: J. Polonsky, *Planta Med.*, Suppl., 107 (1966)

[7] Z. Valenta, S. Papadopoulos and C. Podeška, *Tetrahedron* **15**, 100 (1961); Z. Valenta, A. H. Gray, D. E. Orr, S. Papadopoulos and C. Podeška, *Ibid.* **18**, 1433 (1962); W. A. C. Brown and G. A. Sim, *Proc. Chem. Soc.* 293 (1964)

[8] C. G. Casinovi, V. Bellavita, G. Grandolini and P. Ceccherelli, *Tetrahedron Letters* 2273 (1965)

[9] [a] H. Hikino, T. Ohta and T. Takemoto, *Chem. Pharm. Bull. Tokyo* **18**, 219 (1970);
[b] H. Hikino, T. Ohta and T. Takemoto, *Ibid.* **18**, 1082 (1970)