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SESQUITERPENES FROM *ACORUS CALAMUS* L.

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Abstract—Five new sesquiterpenes were isolated from *Acorus calamus* L., in addition to calameone (or calamendiol), the structure of which was revised, and their structures were established. Furthermore, chemical co-relation among these sesquiterpenes was carried out. In particular, the thermal isomerization of shyobunone (I), an elemene-type sesquiterpene, to preisocalamendiol (VI), a germacrone-type compound, should be noted.

STUDIES on sesquiterpenes isolated from sweet flag oil have been carried out by many workers.[1] particularly Sorm *et al.* We have examined sesquiterpenic components of the rhizoma of *Acorus calamus* L. (Japanese name "Shyobu") growing in Japan, and isolated several new sesquiterpenes.[2] We describe the isolation and structures of these new sesquiterpenes.

Two different procedures for isolation of these sesquiterpenes were examined: The ether extract of the steam distillate of the rhizomes was separated by repeated silica gel chromatography to afford three elemene-type sesquiterpenes (I, II and III), asarone and its isomers and two cadinene-type sesquiterpenes (IV and V). In this procedure. isolation of unstable germacrene-or germacrone-type compounds are not always expected. We further examined direct separation of these compounds from *Acorus calamus* L. by preparative GLC: The rhizomes of *Acorus calamus* L. were immersed in methanol. The methanol extract was concentrated under reduced pressure below room temperature. and directly separated by preparative GLC to give preisocalamendiol (VI). a germacrone-type sesquiterpenes.

Comparison of gas-chromatograms between the methanol extract and the ether extract of steam distillate.

Gas-chromatograms of these extracts are shown in Fig. 1. In the comparison of GLC, most of peaks are common to both, but peak D corresponding to preisocalamendiol (VI) is weaker in the latter. During steam distillation. preisocalamendiol (VI) is probably converted into the others. For example, the possibility that isocalamendiol (IV) is an artifact cannot be ruled out. In fact. VI was converted into isocalamendiol (IV). a cadinene-type sesquiterpene. when treated with 80% acetic acid.[3]

Steam distillation is convenient for the isolation of elemene-type sesquiterpenes (I. II and III) as well as cadinene-type compounds (IV and V). From these compounds (I. II. III and IV) germacrone-type compounds (VI and VIII) have been synthesized for biogenetic-type reactions.[4]

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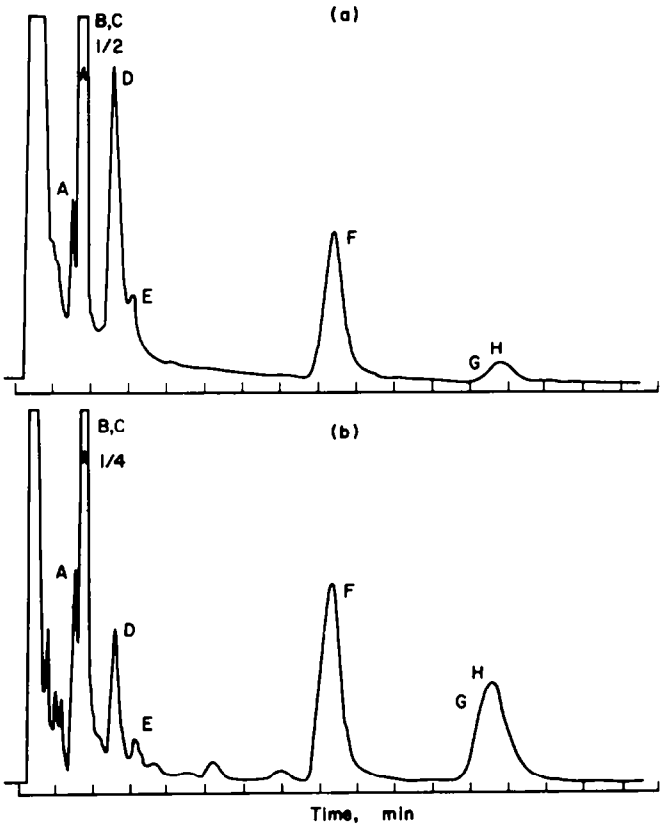


FIG 1. (A) Gas-chromatogram of the methanol extracts.

FIG 1. (B) Gas-chromatogram of the ether extracts of steam distillate.

A: Epishyobunone (II), B: Isoshyobunone (III), C: Shyobunone (I), D: Preisocalamendiol (VI), E: Dehydroxyisocalamendiol (XXII), F: Asarone and its isomers, G: Calameone (V), H: Isocalamendiol (IV). (5% PEG 20M on Celite 545 (100 mesh) at 170°).

Structures of shyobunone (I), *epishyobunone* (II) *and isoshyobunone* (III).[2a]

Shyobunone, epi- and isoshyobunone are colourless liquids, all of which have the same molecular formula ($C_{15}H_{24}O$). The IR and UV spectra of shyobunone (I) coupled with its NMR spectrum (experimental) indicate the presence of a CO group and more than two double bonds. In fact, catalytic hydrogenation of I afforded a tetrahydro derivative (VIII), indicating the presence of two double bonds in the original ketone (I). Furthermore, shyobunone gave a mixture of two hydroxy derivatives (IX and X) in 58 and 9% yields respectively, when treated with LAH. This evidence indicates that shyobunone, which has two secondary Me's, a tertiary Me and a Me group attached to a double bond (experimental), is a monocyclic elemene-type sesquiterpene. Accordingly, a tentative structure (A) was given to shyobunone except for position of the CO group which must be located at one of three possible positions (†). Furthermore, the NMR signal at δ 2·95 ppm (1H, s) and co-occurrence of isoshyobunone (III) having an $\alpha\beta$-unsaturated CO group suggest that the CO group is located at the asterisk-position. Finally, the structure of shyobunone was represented

by I. which proved to be identical with 2β-isopropenyl-3β-methyl-3α-vinyl-6β-isopropylcyclohexanone chemically transformed from costunolide (XI) (IR and NMR spectra).[5]

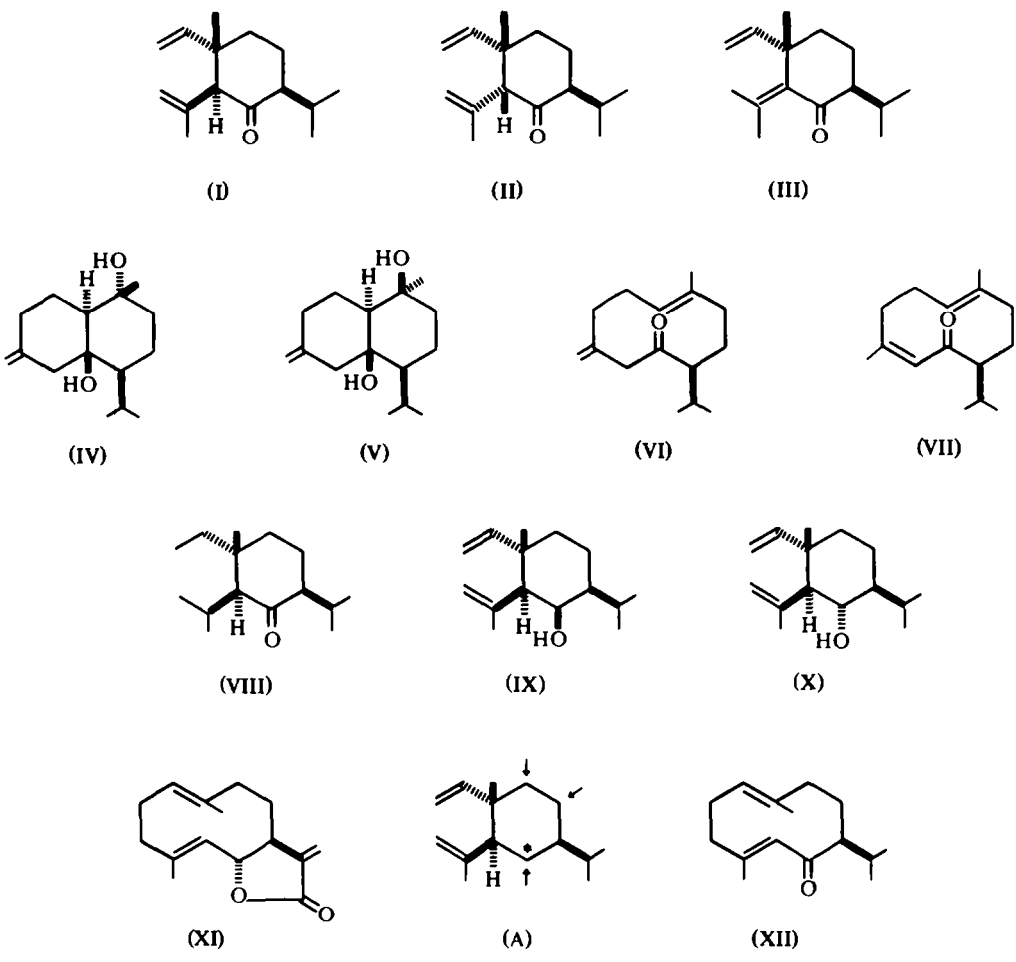


FIG. 2

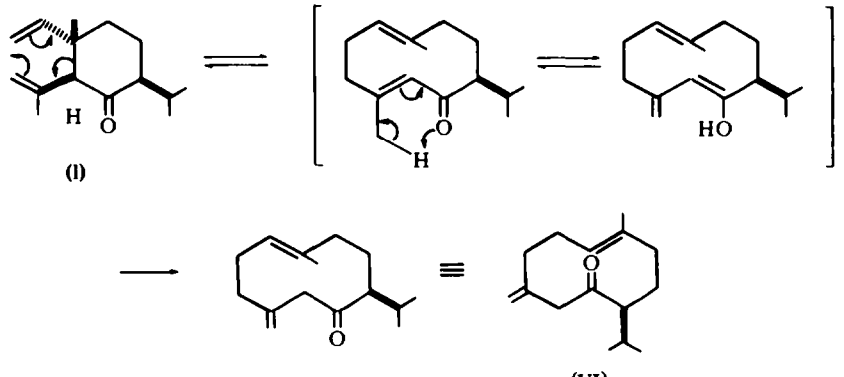
Epishyobunone (II) obtained from the second fraction of silica gel chromatography has a mass spectrum very close to that of shyobunone (I) except for a peak at *m/e* 192 ($M^+ - 28$). In addition. its IR. UV and NMR spectra are also very similar to that of I. Therefore. epishyobunone is considered to be a stereo-isomer of shyobunone. In fact. the ORD curve of shyobunone (I) showed a positive Cotton effect ($[\phi]^{P}_{318} + 18{\cdot}7° \times 10^2$. $[\phi]^{T}_{275} - 121° \times 10^2$. A = + 140) which is in good agreement with the expected configuration (I). whereas that of epishyobunone (II) had a strongly negative Cotton effect ($[\phi]^{T}_{315} - 89° \times 10^2$. $[\phi]^{P}_{279} + 111° \times 10^2$. A = −200). indicating that the latter has an axial isopropenyl group at C_2-position.

The IR and UV spectra of isoshyobunone coupled with the NMR signals at δ 1·75 ppm (6H, s) satisfied the structure of an αβ-unsaturated cisoid ketone (III). In fact, isoshyobunone (III) is readily obtained by treatment of both unconjugated ketones (I and II) with sodium methoxide or 100% formic acid. Furthermore, thermal isomerizations of both I and II into III have been unsuccessful → successful.[4] These results establish the structural relationship between these three sesquiterpenes (I, II and III).

From a biogenetic point of view, isoshyobunone (III) is probably transformed from I or II. Furthermore, such a germacrone-type compound as XII is regarded as a precursor of elemene-type sesquiterpenes (I and II). Accordingly, our efforts were focused on the isolation of the biogenetically important precursor (XII) from the plant, and we isolated preisocalamendiol (VI), a germacrone-type sesquiterpene, instead of XII.

Structure of preisocalamendiol (VI)

Preisocalamendiol (VI) is a monocyclic ketone having a molecular formula ($C_{15}H_{24}O$). The IR and NMR spectra indicate the presence of an exocyclic double bond (>C=CH_2). The NMR signals at δ 0·86 (3H, d, J = 6·7 Hz) and 0·91 (3H, d, J = 6·7 Hz), and 1·37 (3H, br.s) and 4·9–5·3 ppm (1H, br.) are assigned to an isopropyl group and a Me group attached to the trisubstituted double bond, respectively. Furthermore, the remarkable AB-quartet is attributable to the methylene group between a CO group and an exocyclic double bond in the tentative structure VI. Finally, the stereostructure of preisocalamendiol (VI) was established by the thermal isomerization of shyobunone (I): Thermal rearrangements of shyobunone (I), epi- and isoshyobunone (II and III) have been investigated under various conditions.[4] When treated in a sealed tube at 160–165°, shyobunone (I) afforded a mixture of several products, which were purified by preparative GLC to give, in 28% yield, preisocalamendiol (VI) in a pure state. The structure of preisocalamendiol (VI) was elucidated by its spectral data coupled with the reaction mechanism, as shown.[4] Biogenetically, preisocalamendiol (VI) must be a precursor of cadinene-type sesquiterpenes (IV and V).

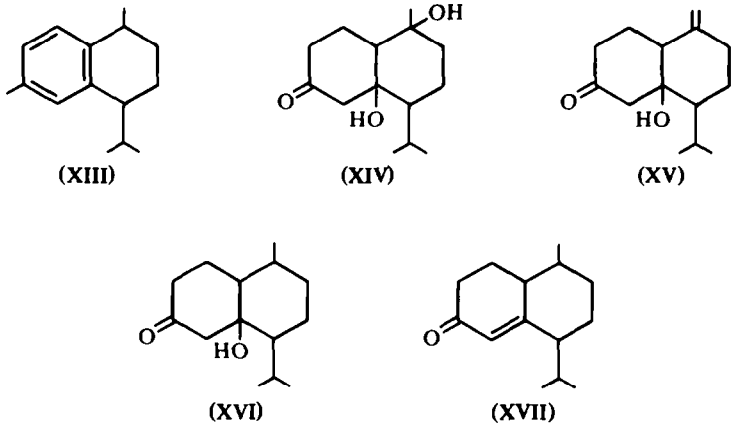


Thermal Isomerization of Shyobunone (I)

Structure of isocalamendiol

Isocalamendiol (IV) has the same molecular formula ($C_{15}H_{26}O_2$) as calameone (or calamendiol).[6] Furthermore, the physical data of both compounds are fairly close. Isocalamendiol has two tertiary OH groups, which cannot be acetylated with acetic anhydride. In addition, the presence of an exocyclic double bond is supported by its IR and NMR spectra. When heated with 100% formic, IV afforded calamenene (XIII) in *ca* 50% yield. Accordingly, isocalamendiol (IV) is regarded as a decahydronaphthalene having two tertiary OH groups and an exocyclic double bond. In the next step, positions of these three functional groups were determined: Ozonolysis of isocalamendiol gave a dihydroxy-ketone (XIV); which was further treated with $POCl_3$–pyridine to afford, in 70% yield, a dehydroxy-ketone (XV). $C_{14}H_{22}O_2$. Formation of a new exocyclic double bond (in XV) was confirmed by the appearance of NMR signals at δ 4·73 (1H, br.s) and 5·00 ppm (1H, br. s) instead of the Me singlet at δ 1·25 ppm (in XIV), and also supported by catalytic hydrogenation of XV which afforded a dihydro-ketone (XVI). These facts indicate that an equatorial OH group must be attached to the C atom bearing a tertiary Me group.

When heated with NaOMe—MeOH under reflux, dihydro-ketone (XVI) afforded, in *ca* 30% yield, an αβ-unsaturated ketone (XVII), $C_{14}H_{22}O$, the structure of which was confirmed by its physical data: The presence of an αβ-unsaturated ketone having one vinyl proton is supported by its IR and UV spectra coupled with the appearance of a singlet at δ 5·84 ppm (1H, s) in the NMR spectrum. Therefore, the exocyclic double bond and one of the two tertiary OH groups in isocalemendiol can be located at C_3 and C_5-positions, respectively. Furthermore, the remaining equatorial OH group must be attached to C_9-carbon atom.



(XIII) (XIV) (XV) (XVI) (XVII)

FIG. 4

Stereochemostry of isocalamendiol

Catalytic hydrogenation of isocalamendiol (IV) afforded a mixture of dihydro-isomers (XVIII) which was directly treated with $POCl_3$–pyridine to give, in almost quantiative yield, a mixture of two dihydro-dehydroxy isomers [XIXa (87%) and XIXb (13%)], which could be separated by preparative GLC. In a comparison of the

NMR spectra of XIXa and XIXb. the Me doublet at δ 1·16 ppm in the former is 0·26 ppm in a lower than the corresponding signal of the latter. The other signals are nearly identical in both compounds. This indicates that XIXa has a 1.3-diaxial relationship between the secondary Me group and the tertiary OH group at C_5-position.[7] Ozonolysis of XIXa followed by decomposition with dimethyl sulfide afforded an oxidation product (XX). $C_{14}H_{24}O_2$. the ORD curve of which showed a negative Cotton effect ($[\phi]^T_{301} - 35{\cdot}4 \times 10^2$. $[\phi]^P_{264}$ $24{\cdot}6 \times 10^2$. $A = -60$). The ketone (XIV) also has a negative Cotton effect curve ($[\phi]^T_{308} - 89{\cdot}1° \times 10^2$. $[\phi]_{290}$ $67{\cdot}2° \times 10^2$. $A = -156$). These facts indicate that the stereo-structure of isocalamendiol can be represented by XXI. Finally. the remaining configuration of an isopropyl group at C_6-position was established by the chemical correlation between isocalamendiol and shyobunone (I): When treated with $POCl_3$- pyridine isocalamendiol gave. in 67% yield. a dienol (XXII). This compound was also obtained by the thermal isomerization of shyobunone (I).[4] the structure of which was known. Therefore. the stereo-structure of isocalamendiol should be represented by IV.

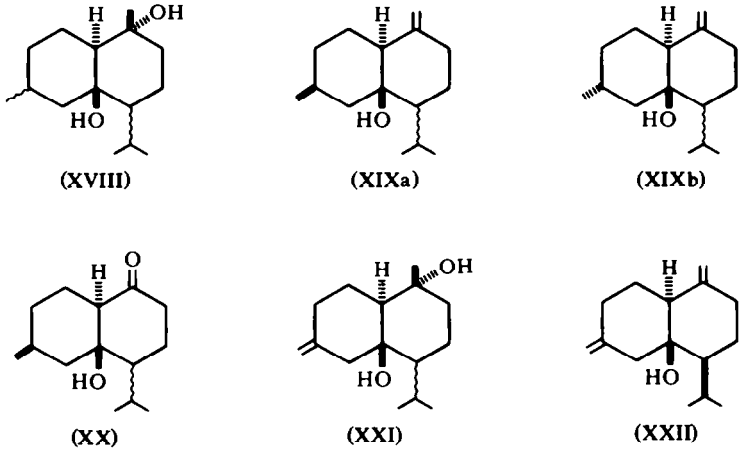


FIG. 5

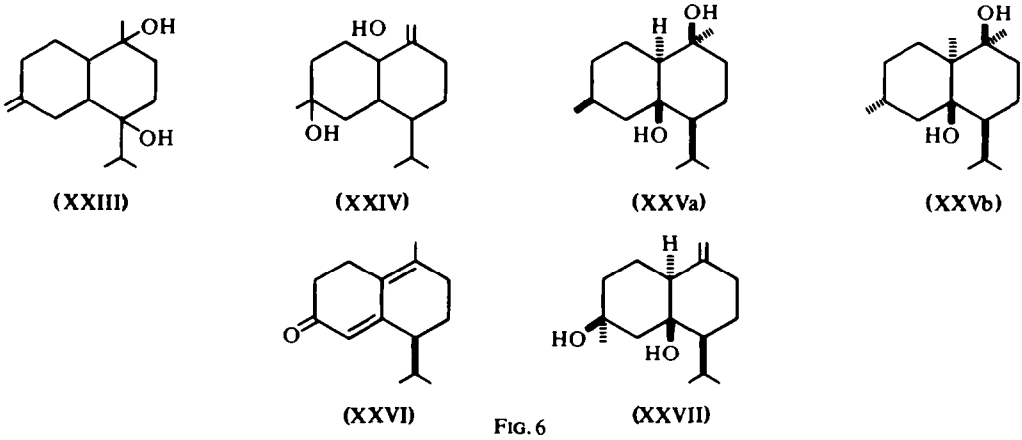
Structure of calameone (calamendiol)

Calameone. m.p. 170·5–171·5° (lit. 169°). has IR and NMR spectra similar to those of isocalamendiol (IV). From these spectral properties, two tentative structures (XXIII and XXIV) cannot be ruled out.[6, 8]

Catalytic hydrogenation of calameone (V) afforded a mixture of two dihydrocompounds (XXVa. m.p. 123–124°. and XXVb. m.p. 133–134°. in 27 and 59% yields. respectively). The former was identical with an authentic sample of dihydrocalameone (m.p. and IR spectrum).[9] whereas the latter showed the same m.p. as reported by Böhme.[10] Each configuration of the newly formed secondary Me groups in two isomers was determined by the comparison of the NMR spectra; XXVa has a Me doublet at δ 1·18 ppm. whereas in XXVb it appears at δ 0·89 ppm. This indicates that the secondary Me group in XXVa should have an axial configuration.[7]

Ozonolysis of isocalamendiol (IV) followed by dehydration gave an oily conjugated ketone (XXVI). the structure of which was confirmed by the UV and NMR

spectra. This compound was also obtained in good yield from calameone, indicating that calameone had the same carbon skeleton as that of IV and an exocyclic double bond at C_3-position. Finally, the structure of calameone was established: The dienol (XXII), which was obtained from isocalamendiol (IV), was oxidized with *m*-chloroperbenzoic acid (1·2 eq.), and then reduced with LAH to afford, in 44% yield, a mixture of two diols [V and XXVII (relative ratio 1 : 1)]. In the above experiments, the peroxyacid can be expected to attack the exocyclic double bond at C_3 or C_9-carbon atom from the same side as that of the tertiary OH group.[11] Fortunately, one of them was completely identical with calameone (m.p. and IR spectrum), the formation of which indicates that the stereo-structure of calameone should be represented by V.

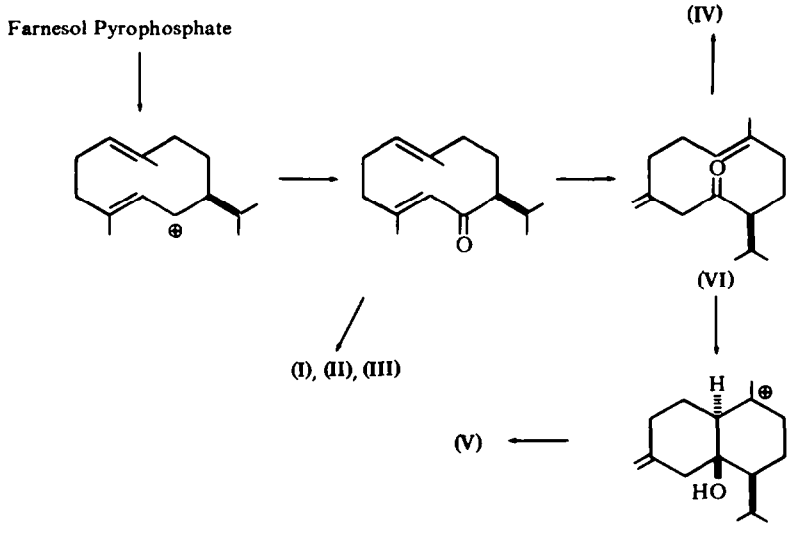


(XXIII) (XXIV) (XXVa) (XXVb)

(XXVI) (XXVII)

FIG. 6

From a biogenetic point of view, preisocalamendiol (VI) is regarded as a precursor of these cadinene-type sesquiterpenes (IV and V). In fact, preisocalamendiol (VI) was readily converted into isocalamendiol (IV) in 47% yield, when treated with 80% acetic acid.



Biogenesis of Sesquiterpenes of *Acorus calamus* L.

EXPERIMENTAL

M.ps are uncorrected. Relative retentions *vs* calamenene as an internal standard were recorded on Shimadzu GC-1C (flame-ionizer detector). Stationary phase: 5% PEG 20M on Celite 545 (100 mesh); Column temp [ϕ 3 mm × 1·5 m (stainless): 110°; Carrier gas: Nitrogen (50 ml/min); Inlet pressure: 1·5 Kg/cm^2]. unless otherwise stated. IR spectra were recorded on JASCO IR-S and Hitachi 215-spectrophotometers. UV spectra were taken on Hitachi-124 and Beckman DK-2 spectrophotometers using MeOH as solvent. NMR spectra were recorded on a Nihondenshi JNM-C60H (60Mc) and a Varian A-60 NMR-spectrometer (60Mc) using $CDCl_3$ as solvent. unless otherwise stated. Only prominent peaks are cited. Chemical shifts are given in ppm relative to internal TMS. Coupling constants are given in Hz (s. singlet; d. doublet; t. triplet; q. quartet; m. multiplet). Mass spectra were obtained on a Hitachi RMU-6D mass spectrometer operating with an ionization energy (70eV). ORD curves were recorded on a JASCO ORD/UV-5 spectrometer using methanol as a solvent. TLC and column chromatography were carried out on Wako gel (B-5 and B-5F) [or alumina (Merck. nach Stahl)] and silicic acid (Mallincklodt. 100 mesh] [or basic alumina (E. Merck. A. G. Germany)] respectively.

Isolation of sesquiterpenes from Acorus calamus L.

Steam distillation of sliced rhizomes (8·0 Kg) of *Acorus calamus* L. growing in Aichi-ken, Japan, was carried out. and then the distillate was extracted with large amounts of ether. The ether extract was dried over Na_2SO_4 and concentrated to afford a pale yellow oil (110 g). The oil (30 g) was chromatographed on silica gel (450 g) and eluted with light petroleum–ether (4:1) to give. in the following order, a mixture of elemene-type compounds (I. II and III) (7·7 g). asarone and its isomers (3·8 g). calameone (0·45 g) and isocalamendiol (2·1 g). The mixture (1 g) was rechromatographed on silica gel (80 g) and eluted with benzene to afford. in the following order. shyobunone (0·75 g). epishyobunone (0·15 g) and isoshyobunone (50 mg). Physical data of these sesquiterpenes are described below.

Shyobunone (I) relative retention. 1·33; ν_{max}(film) 3080. 1710. 1639. 911 and 891 cm^{-1}; λ_{max} 206 mμ (ε. 3010); δ (CCl_4) 0·87 (3H. d. J = 6·4 Hz). 0·90 (3H. d. J = 6·4 Hz). 1·02 (3H. s). 1·76 (3H. br.s). 2·95 (1H. s). 4·65–5·10 (4H. m) and 5·82 ppm (1H. q. J = 18. 10 Hz) (Found: M$^+$ = 220·1827; $C_{15}H_{24}O$ requires: M$^+$ = 220·1827).

Epishyobunone (II). relative retention. 1·07; ν_{max}(film) 3080. 1710. 1641. 910 and 890 cm^{-1}; λ_{max} 207 mμ (ε. 3400); δ (CCl_4) 0·85 (3H. d. J = 6·4 Hz). 0·98 (3H. d. J = 6·4 Hz). 1·05 (3H.s). 1·77 (3H. br.s). 3·00 (3H. s). 4·02–5·07 (4H. m) and 5·72 ppm (1H. q. J = 18. 10 Hz). (Found: M$^+$ = 220·1840; $C_{15}H_{24}O$ requires: M$^+$ = 220·1827).

Isoshyobunone (III). relative retention. 1·23; ν_{max}(film) 1678. 1640 and 1614 cm^{-1}; λ_{max} 252 mμ (ε. 4180); δ (CCl_4) 0·85 (3H. d. J = 6·4 Hz). 0·89 (3H. d. J = 6·4 Hz). 1·36 (3H. s). 1·75 (6H. s). 4·95 (1H. q. J = 18. 1·5 Hz). 4·96 (1H. q. J = 10. 1·5 Hz) and 5·94 ppm (1H. q. J = 18. 10 Hz). (Found: M$^+$ = 220·1829; $C_{15}H_{24}O$ requires: M$^+$ = 220·1827).

Calameone (or *Calamendiol*) (V). m.p. 170·5–171·5° (from n-hexane–ether) (lit.. 169°);[6] ν_{max}(KBr) 3370 br.. 3080 and 1650 cm^{-1}; δ 0·90 (6H. d. J = 6·8 Hz). 1·13 (3H. s). 2·87 (2H. s) 4·80 (1H. br.s) and 4·89 ppm (1H. br.s): *m/e* 238 (M$^+$) (Found: C. 75·58; H. 11·20. $C_{15}H_{26}O_2$ requires: C. 75·58; H. 11·00%).

Isocalamendiol (IV). m.p. 72·5–73·5° (from n-hexane–ether); ν_{max}(KBr) 3600. 3380 br.. 3090 and 1648 cm^{-1}; δ 0·90 (3H. d. J = 7 Hz). 0·93 (3H. d. J = 7 Hz). 1·22 (3H. s). 4·80 (1H. br.s) and 4·89 ppm (1H. br.s); *m/e* 238 (M$^+$). (Found: C. 75·64; H. 10·92. $C_{15}H_{26}O_2$ requires: C. 75·58; H. 11·00%).

Isolation of preisocalamendiol (VI)

Sliced rhizomes (*ca* 35 g) were immersed in MeOH (*ca* 300 ml) for a month at room temp. and then the extract was evaporated under reduced pressure below 25° to leave a brown oil. from which the fraction corresponding to the peak D (see the Fig 1 (A)) was separated by preparative GLC (20% DEGS on Celite 545 (80–100 mesh) at 110°) to give VI in a pure state (*ca* 10% of the MeOH extract).*

Preisocalamendiol (VI). relative retention. 2·24; ν_{max}(film) 3080. 1705. 1642 and 897 cm^{-1}; δ (CCl_4) 0·86 (3H. d. J = 6·7 Hz). 0·91 (3H. d. J = 6·7 Hz). 1·37 (3H. br.s). 2·83 (1H. d. J = 15 Hz). 3·24 (1H. d. J = 15 Hz). 4·84 (1H. br.s). 4·95 (1H. br.s) and 4·9–5·3 ppm (1H. br.m); *m/e* 220 (M$^+$). 205 and 202 (M$^+$–18). (Found: M$^+$ = 220·1813; $C_{15}H_{24}O$ requires: M$^+$ = 220·1827).

* This procedure is not always satisfactory. Further details will be reported together with other isolation procedures such as $AgNO_3$-complex method.[12] Isolation of XII. which must be present in the plant. has been attempted continuously.

Tetrahydroshyobunone (VIII)

Catalytic hydrogenation of shyobunone (474 mg) in EtOAc (10 ml) over PtO_2 (9 mg) was carried out at room temp for 2 hr. and then filtered to remove the catalyst. The filtrate was concentrated under reduced pressure to give colourless liquid in almost quantitative yield. which was purified by preparative GLC (20% PEG 20M on Celite 545 (80–100 mesh) at 110°). relative retention. 0·73; ν_{max}(film) 1705 cm^{-1}; δ (CCl_4) 0·75–1·00 (15H. complex) and 1·11 ppm (3H. d. J = 7·0 Hz). (Found: M^+ = 224·2207; $C_{15}H_{28}O$ requires: M^+ = 224·2140).

Reduction of shyobunone (I) *with lithium aluminium hydride*

To a soln of shyobunone (102 mg) in dry ether (5 ml). dry ether (20 ml) containing LAH (101 mg). was carefully added with stirring. The mixture was stirred at room temp overnight. and then decomposed with water. made acidic with 5% HCl aq and then extracted with ether. The ether extract was washed with water. and then dried over $MgSO_4$. Removal of the solvent afforded an oil. which was chromatographed on silica gel (10 g) and eluted with chloroform to give a colourless liquid (60 mg) of IX as the first fraction. After elution of IX. a colourless liquid (*ca* 9 mg) of an isomer (X) was isolated. the IR spectrum of which was identical with that of XV.[5] Physical data of these reduction products are described below.

Shyobunol (IX). relative retention. 1·63; ν_{max}(film) 3600. 3090. 1640 and 895 cm^{-1}; δ 0·95 (6H. d. J = 6·2 Hz). 1·31 (3H. s). 1·79 (3H. br.s). 3·8–3·95 (1H. m). 4·65–5·05 (4H. complex) and 5·75 ppm (1H. q. J = 17·5. 10 Hz); *m/e* 222 (M^+). 207. 204. 190. 179 and 161. (Found: M^+ = 222·1958; $C_{15}H_{26}O$ requires: M^+ = 222·1984).

An isomer of shyobunol (X). relative retention. 1·32; *m/e* 222 (M^+). 207. 204. 190. 179 and 161. (Found: M^+ = 222·1959; $C_{15}H_{26}O$ requires: M^+ = 222·1984).

Isomerization of shyobunone (I) *into isoshyobunone* (III)

A soln of shyobunone (0·75 g) in abs MeOH (10 ml) containing NaOMe* was heated on a water bath for 18 hr. and then cooled to room temp. The soln was diluted with water and made acidic with dil HCl. and then extracted with ether. The ether extract was dried over Na_2SO_4 and evaporated to leave a yellow oil (0·5 g). which was purified by preparative GLC (14% PAG 20M on Celite 545 (80–100 mesh) at 130°) to give III (GLC and IR spectrum).

Isomeriaztion of epishyobunone (II) *into isoshyobunone* (III)

A soln of epishyobunone (170 mg) in abs MeOH (10 ml) containing NaOMe* was heated under the same conditions as that of I. and worked up to afford a yellow oil (85 mg). which was also purified by preparative GLC to give III (GLC and IR spectrum).

Rearrangements of shyobunone (I) *and epishyobunone* (II) *into isoshyobunone* (III)

(i) A soln of shyobunone (0·54 g) in 100% formic acid (16 g) was heated under reflux for 1 hr. and then extracted with ether. The ether extract was washed well with water. dried over $MgSO_4$. and then concentrated to leave an oil (0·4 g) which was purified by preparative GLC [30% PEG 20M on Celite 545 (80–100 mesh) at 130°] to give III (GLC and IR spectrum).

(ii) A soln of epishyobunone (10 mg) in 100% formic acid (0·5 g) under the same conditions gave a small amount of III (GLC and IR spectrum).

Thermal isomerization of shyobunone (I) *to preisocalamendiol* (VI)

Shyobunone (245 mg) was heated in a sealed tube at 160–165° for 20 min. and then the mixture was separated directly by preparative GLC (20% DEGS on Celite 545 (80–100 mesh) at 110°) to give a colourless liquid (70 mg).[4] which was identical with VI (GLC and IR spectrum). Accordingly. VI. which has been used for biogenetic-type reactions. has been synthesized from I without direct isolation from the plant.

Dehydration of isocalamendiol (VI) *with 100% formic acid*

Isocalamendiol (222 mg) was treated with 100% formic acid (2 ml) according to Treibs' procedure[6] to give 182 mg of a crude oil. which was chromatographed on silica gel (20 g) and eluted with n-hexane to give an almost colourless liquid (93 mg). Preparative TLC (silica gel) using n-hexane afforded a colourless liquid (28 mg). which was identical with an authentic sample of calamenene (IR and NMR spectrum).[13]

* The methanolic solution was prepared from Na (2·5 g) and abs. methanol (40 ml).

Ozonolysis of isocalamendiol

According to Pappas' procedure.[14] a soln of isocalamendiol (565 mg) in MeOH (20 ml) was ozonized at −70° for 1·5 hr. and then treated with 2 ml dimethyl sulfide to give crude crystals, which were recrystallized from n-hexane–ether to give 366 mg of colourless prisms (XIV), m.p. 145–146°; ν_{max}(KBr) 3480 br. and 1702 cm^{-1}; δ 0·89 (3H. d. J = 6·8 Hz). 0·95 (3H. d. J = 6·8 Hz) and 1·25 ppm (3H. s); m/e 222 (M^+−18). 207 and 179. (Found: C. 70·13; H. 10·06. $C_{14}H_{24}O_3$ requires: C. 69·96; H. 10·07%).

Dehydration of ketone (XIV) *with* $POCl_3$*-pyridine*

To a soln of the XIV (426 mg) in pyridine (8·5 ml), 0·7 ml of $POCl_3$ at −15°, was slowly added and then the temperature was gradually elevated to room temp. The soln was stirred at room temp for 40 hr. and then poured into ice-water and extracted with ether. The ether extract was washed with sat NaCl aq and dried over $MgSO_4$. Removal of the solvent gave an oil which was dissolved in n-hexane at 0° to leave 288 mg of colourless needles (XV), m.p. 119–119·5° (from CCl_4); ν_{max}(KBr) 3420, 1705 and 1640 cm^{-1}; δ 0·88 (3H. d. J = 6·9 Hz). 0·93 (3H. d. J = 6·9 Hz). 1·31 (1H. s. OH). 4·73 (1H. br.s) and 5·00 ppm (1H. br.s); m/e 222 (M^+). 207 and 204 (Found: C. 75·09; H. 10·14 (M^+ = 222·1571). $C_{14}H_{22}O_2$ requires: C. 75·63; H. 9·97% (M^+ = 222·1620).

Catalytic hydrogenation of dehydroxy-ketone (XV)

A mixture of the dehydroxy-ketone (40 mg) and PtO_2 (5 mg) in EtOAc (5 ml) was stirred at room temp for 50 min under H_2. and then filtered. The filtrate was concentrated under reduced pressure to leave white crystals. which were recrystallized from n-hexane to give 32 mg of XVI. m.p. 124·5–126·5°; ν_{max}(KBr) 3550, 3460br. and 1705 cm^{-1}; δ 0·80–1·10 (9H, complex) and 1·48 ppm (1H, br.s, OH); m/e 224 (M^+), 209 and 206 (Found: M^+ = 224·1700; $C_{14}H_{24}O_2$ requires: M^+ = 224·1776).

Formation of αβ*-unsaturated ketone* (XVII)

(i) To a soln of the dihydro-ketone (60 mg) in MeOH (8 ml), a soln of NaOMe in MeOH (1 ml) was added*. The soln was heated under reflux for 12 hr, and then poured into water and extracted with ether. The ether extract was washed with water and dried over $MgSO_4$. Removal of the solvent gave 20 mg of an yellow oil. which was purified by preparative TLC (silica gel) using benzene as a solvent to give colourless liquid. relative retention *vs* calamenene, 4·62 (at 150°; Inlet pressure: 1·8 Kg/cm^2); ν_{max} (film) 1679 and 1617 cm^{-1}; ν_{max}243 mμ (ε, 12660); δ 0·78 (3H, d, J = 6·7 Hz), 0·9–1·1 (6H, m) and 5·84 ppm (1H, s); m/e 206 (M^+). 191. 178. 164 and 163. (Found: M^+ = 206·1670; $C_{14}H_{22}O$ requires: M^+ = 206·1671).

(ii) A soln of the dihydro-ketone (30 mg) in 100% formic acid (1·5 ml) was heated under reflux for 30 min (under N_2), and then cooled to room temp. After addition of water, the soln was made slightly basic with Na_2CO_3 and extracted with ether. The ether extract was washed with sat NaCl aq and then dried over $MgSO_4$. Removal of the solvent afforded 23 mg of a brown oil, which was purified by repeated preparative TLC (1. silica gel in light petroleum–ether (7:1); 2. alumina in light petroleum–ether (1:1)) to give 14 mg of colourless liquid (XVII) (GLC and IR spectrum).

Catalytic hydrogenation of isocalamendiol (IV)

Catalytic hydrogenation of isocalamendiol (800 mg) in EtOAc (10 ml) was carried out over PtO_2 (14 mg) at room temp for 3 hr. After filtration of the catalyst. the solvent was evaporated to leave 800 mg of XVIII. m.p. 85–86° (from n-hexane); ν_{max}(KBr) 3650 and 3400 br. cm^{-1}; δ 0·88 (3H, d, J = 6·9 Hz), 0·92 (3H, d, J = 6·9 Hz). 1·05–1·28 (3H. complex) and 1·24 ppm (3H, s); m/e 240 (M^+). (Found: C, 74·38; H. 12·16. $C_{15}H_{28}O_2$ requires: C, 74·95; H, 11·74%). This material thus obtained was a mixture of two isomers, which were not separated and directly used for the next experiment.

Dehydration of the above reaction mixture (XVIII) *with* $POCl_3$*-pyridine*

To a soln of XVIII (750 mg) in pyridine (15 ml) 1·3 ml of $POCl_3$ at −20°, was added slowly and then the temp was elevated to room temp. The soln was allowed to stand at room temp for 12 hr. and then poured into ice-water and extracted with ether. The ether extract was washed with water and then dried over $MgSO_4$. Removal of the solvent gave 677 mg of pale yellow liquid, GLC of which showed two peaks (XIXa, 87%; XIXb. 13%). The mixture was separated by preparative GLC [25% DEGS on Celite 545 (60–80 mesh) at 110°]. Physical data of both compounds are described below. XIXa: relative retention *vs* cyclohexanone, 10·5;

* The methanolic soln was prepared from Na (1 g) and abs MeOH (40 ml).

ν_{max}(film) 3583 and 1650 cm^{-1}; δ 0·85 (3H, d, J = 7·3 Hz), 0·88 (3H, d, J = 7·3 Hz), 1·16 (3H, d, J = 7·1 Hz), 4·68 (1H, br.s) and 4·89 ppm (1H, br.s); m/e 222 (M^+), 207 and 204 (Found: M^+ = 222·1967; $C_{15}H_{26}O$ requires: M^+ = 222·1984).

Compound XIXb, relative retention *vs* cyclohexanone, 13·4; ν_{max} (film) 3590 and 1650 cm^{-1}; δ 0·86 (3H, d, J = 8 Hz), 0·90 (3H, d, J = 6·8 Hz), 0·90 (3H, d, J = 6·6 Hz), 4·65 (1H, br.s) and 4·89 ppm (1H, br.s); m/e 222 (M^+), 207 and 204 (Fpund: M^+ = 222·1973; $C_{15}H_{260}$ requires: M^+ = 222·1984).

Ozonolysis of XIXa

According to Pappas' procedure,[14] a soln of XIXa (48 mg) in MeOH (20 ml) was ozonized at −70° for 1 hr, and then treated with dimethyl sulfide (0·5 ml) to give white crystals which were chromatographed on silica gel (1·7 g) and eluted with ether to afford 46 mg of white crystals (XX), m.p. 145–146° (from ether); ν_{max}(KBr) 3420 and 1700 cm^{-1}; δ 0·91 (3H, d, J = 6·9 Hz), 0·98 (3H, d, J = 6·9 Hz), 1·14 (3H, d, J = 7·2 Hz) and 1·45 ppm (1H, s, OH); m/e 224 (M^+) and 206 (Found: C, 74·37; H, 10·52. $C_{14}H_{24}O_2$ requires: C, 74·95; H, 10·78%).

Dehydration of isocalamendiol (IV) *with* $POCl_3$*-pyridine*

To a soln of isocalamendiol (820 mg), 1·0 ml of $POCl_3$ was slowly added at −17°. The soln was allowed to stand at 0° for 2 hr, and then poured into ice-water and extracted with ether. The ether extract was washed with water, and then dried over $MgSO_4$. Removal of the solvent gave 690 mg of pale yellow liquid, which was mixed with 2·8 g silica gel and then put on 20 g silica gel. Elution with benzene gave 500 mg colourless liquid (XXII), relative retention, 2·91; ν_{max}(film) 3550, 3080, 1645 and 890 cm^{-1}; δ 0·90 (3H, d, J = 6·7 Hz), 0·93 (3H, d, J = 6·7 Hz), 4·60 (1H, br.s) and 4·7–4·9 ppm (3H, br.m) (Found: M^+ = 220·1811; $C_{15}H_{24}O$ requires: M^+ = 220·1827).

Catalytic hydrogenation of calameone (V)

Catalytic hydrogenation of calameone (81 mg) in EtOAc (8 ml) was carried out over PtO_2 (11 mg) at room temp for 3 hr. After filtration of the catalyst, the solvent was evaporated under reduced pressure to give a white crystalline solid, which was chromatographed on silica gel (2·5 g) and eluted with light petroleum–ether (2:1) to give 22 mg of colourless needles (XXVa), m.p. 123–124° (from n-hexane); ν_{max}(KBr) 3300 br. cm^{-1}; δ 0·91 (6H, d, J = 6·7 Hz), 1·15 (3H, s), 1·18 (3H, d, J = 7·0 Hz) 2·60 ppm (2H, br.s); m/e 240 (M^+) (Found: C, 74·79; H, 11·85. $C_{15}H_{28}O_2$ requires: C, 74·95; H, 11·74%). Further elution with the same solvent afforded 48 mg of XXVb, m.p. 133–134° (from n-hexane); ν_{max}(KBr) 3320 br. cm^{-1}; δ 0·89 (3H, d, J = 6·0 Hz), 0·92 (6H, d, J = 6·8 Hz), 1·15 (3H, s) and 2·74 ppm (2H, s); m/e 240 (M^+) (Found: C, 74·55; H, 11·79. $C_{15}H_{28}O_2$ requires: C, 74·95; H, 11·74%).

Ozonolysis of calameone (V)

According to Pappas' procedure,[14] a soln of calameone (210 mg) in MeOH (20 ml) was ozonized at −70° for 1 hr, and then treated with dimethyl sulfide (2 ml) to give crude crystals, which were recrystallized from n-hexane–ether to afford 132 mg of prisms, m.p. 179–179·5°; ν_{max}(KBr) 3440, 3390 br. and 1690 cm^{-1}; δ 0·93 (6H, d, J = 6·3 Hz) and 1·26 ppm (3H, s); m/e 222 ($M^+ - 18$) (Found: C, 70·12; H, 10·34. $C_{14}H_{24}O_3$ requires: C, 69·96; H, 10·07%).

Dehydration of dihydroxy-ketone (XIV)

A soln of the dihydroxy-ketone (105 mg) in 100% formic acid (2 ml) was heated under reflux for 30 min, and then cooled to room temp. After addition of water, the soln was made neutral with powdered $NaHCO_3$, and then extracted with ether. The ether extract was washed with sat NaCl aq and then dried over Na_2SO_4. Removal of the solvent gave a pale brown oil, which was purified by preparative TLC (silica gel) using light petroleum–ether (2:1) to afford 54 mg of pale yellow liquid (XXVI), relative retention *vs* calamenene, 9·50 (at 150°; Inlet pressure: 1·8 Kg/cm^2); ν_{max} (film) 1660, 1630 and 1580 cm^{-1}; λ_{max} 209, 237 and 302 mμ (ε, 5700, 4600 and 15600, respectively); δ 0·87 (3H, d, J = 6·0 Hz), 0·98 (3H, d, J = 6·0 Hz), 1·85 (3H, s) and 5·68 ppm (1H, s); m/e 204 (M^+) (Found: M^+ = 204·1530; $C_{14}H_{20}O$ requires: M^+ = 204·1514).

Dehydration of dihydroxy-ketone (from calameone)

A soln of the dihydroxy-ketone (from calameone) (96 mg) in 100% formic acid (2 ml) was heated under reflux for 30 min, and then cooled to room temp. After addition of water, the soln was made neutral with powdered $NaHCO_3$, and then extracted with ether. The ether extract was washed with sat NaCl aq and then

dried over Na_2SO_4. Removal of the solvent afforded a pale yellow oil, which was purified by preparative TLC (silica gel) using light petroleum–ether (2:1) to give 54 mg of pale yellow liquid (XXVI) (GLC and IR spectrum).

Formation of calameone (V) *and its isomer* (XXVII) *from dienol* (XXII)

A soln of the dienol (117 mg) and 75% *m*-chloroperbenzoic acid (150 mg) in ether (10 ml) was heated under reflux for 15 hr, and then cooled to room temp. The soln was successively washed with 10% Na_2SO_3, aq 5% $NaHCO_3$ aq and then sat NaCl aq and dried over Na_2SO_4. Removal of the solvent gave a colourless oil, which was directly used for the next experiment.

To a soln of the above oil in abs ether (10 ml), 10 mg of LAH was added with stirring. The mixture was stirred at room temp for 2 hr, and then decomposed with sat aqueous ether. The etheral soln was washed with 20% sodium potassium tartarate aq and sat NaCl, aq and then dried over Na_2SO_4. Removal of the solvent gave a colourless oil, which was purified by preparative TLC (silica gel) using light petroleum–ether (7:3) to give 28 mg of V (mixed m.p. and IR spectrum). Further elution with the same solvent system afforded 28 mg of isomeric XXVII, m.p. 168·5–170° (from n-hexane–ether); ν_{max} (KBr) 3370 br., 3080 and 1650 cm^{-1}; δ 0·93 (6H, d, J = 6·5 Hz), 1·12 (3H, s) and 4·68–5·00 ppm (2H, br. m); *m/e* 238 (M^+) (Found: C, 75·29; H, 11·20. $C_{15}H_{26}O_2$ requires: C, 75·58; H, 11·00%).

Conversion of preisocalamendiol (VI) into isocalamendiol (IV)

Preisocalamendiol (80 mg) was dissolved in 80% AcOH (5 ml) and stirred at room temp for 5 min, and then diluted with ice-water. The soln was made neutral with $NaHCO_3$ aq and extracted with ether. The ether extract was washed with sat NaCl aq and then dried over Na_2SO_4. Removal of the solvent gave a colourless oil, which was purified by preparative TLC (silica gel) using light petroleum–ether (2;1) to afford 41 mg of white crystals (IV) (m.p. and IR spectrum).

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