Tetrahedron. Vol. 33, pp. 2509 to 2511. Pergamon Press 1977. Printed in Great Britain

STUDIES ON THE SESQUITERPENOIDS OF *HYPOLEPIS PUNCTATA* METT.—I

ISOLATION AND STRUCTURE DETERMINATION OF HYPACRONE, A NEW SECO-ILLUDOID

YUJI HAYASHI,* MUGIO NISHIZAWA and TAKEO SAKAN
Faculty of Science, Osaka City University Sugimotocho, Sumiyoshiku, Osaka 558, Japan

(*Received in Japan* 28 *March* 1977; *Received in the UK for publication* 12 *April* 1977)

Abstract—Hypacrone (1), a new seco-illudoid sesquiterpene, was isolated from a fern, *Hypolepis punctata* Mett., as its characteristic acrid principle. The structure is proposed on the spectral basis and the transformation into pterosins, illudoid constituents of the same plant.

The fresh leaves of *Hypolepis punctata* Mett., a fern of Pteridaceae family (Japanese name: Iwahimewarabi), have strong acrid taste. The chemical studies to establish the constituents of this fern, have resulted in four new sesquiterpenoids. Their structures have been determined by spectral studies and total synthesis.[1] One of them is a desired acrid principle, hypacrone (1), which possesses a unique seco-illudane structure. The structure of hypacrone is formally presented by a different fission of the illudane carbon skeleton from fomannosin[2] (2), a known example of secoilludoid. In this communication the isolation and the structure proposal of hypacrone[1b] are described. The remaining three principles, pterosins, H (7), I (8) and Z (6), were indanone derivatives with a illudane framework.[1a,3] It should be noted that the fern contains a seco-illudoid as well as illudoids[4] as sesquiterpenoid constituents, since most of the analogous illudoids have only been found in fungal plants.[5]

The isolation of the acrid principle was difficult, since the taste of the leaves often became undetectable during the isolation processes, quick air-drying, lyophilization or extraction with organic solvents. Finally, extraction with hot water followed by quick fractionation was found to be effective and the following procedure was established for the isolation of hypacrone.

Fresh shoots of the fern collected in May to June were immediately extracted with hot water for about 30 minutes. Active charcoal chromatography of the aqueous exact, concentrated the acrid substance in an ethanol fraction. A crude principle, which contains a considerable quantity of pterosins, was further fractionated with water on a polyamide-gel column, on which hypacrone was clearly separated from pterosins. After this stage, hypacrone was stable, and could be submitted to preparative layer chromatography and finally to vacuum distillation.

Hypacrone (1) has a conjugated α,β-γ,δ-dienone system, which was proved by the UV absorption max at 285 nm. Hydrogenation of 1 on platinum catalyst gave a tetrahydro-diketone† (3), ν_{max} 1730, 1680 cm^{-1}, and a hexahydro-ketol† (4), ν_{max} 3300, 1685 cm^{-1}, which indicated the presence of an additional CO group other than that of the dienone system. Reduction of 1 with sodium borohydride, forming an unstable diene-diol† (5) which gave a diacetate,† also supported the proposed functional groups.

The PMR spectrum (Fig. 1) revealed the types of protons in hypacrone: a gem-dimethyl group (1.06 ppm, 6H), an allylic methylene group (2.49 ppm), and two olefinic protons (5.82 and 6.22 ppm). A sharp 3-proton singlet at 2.11 ppm and a 3-proton doublet at 2.06 ppm (J = 1.3 Hz) were assigned to an acetyl Me and a vinyl Me group (at C-7), respectively. A 1,1-disubstituted cyclopropane ring was proved by a symmetrical pair of 2-proton multiplets (A_2B_2 type), at 0.95 and 1.51 ppm, which displaced to 0.73 and 1.11 ppm in the spectrum of the tetrahydro-diketone (3). The chemical shifts of these 4-proton signals are recorded at relatively lower field than those of usual cyclopropane derivatives, even likewise in the tetrahydro-compound (3); this fact possibly demonstrates the geminal substitution of two unsaturated groups, the acetyl group and the dienone group.

The PMDR examination disclosed the interactions between the Me protons (2.06 ppm) and one olefinic proton (6.22 ppm), and the methylene protons (2.49 ppm) and the other olefinic proton (5.82 ppm); both couples should locate in allylic relationship (J = 1.3 and 1.5 Hz, respectively). Little interaction was observed between both olefinic protons. The rather simple pattern of the individual PMR signals may suggest a symmetrical property of the hypacrone molecule.

Proof of the gross structure and the configuration of the 6,7-double bond were provided by the transformation of hypacrone into pterosins. When hypacrone was heated with aqueous sulfuric acid in tetrahydrofuran solution, pterosin Z (6) was produced as a sole crystalline product in good yield, while aqueous hydrochloric acid gave a chlorine-containing pterosin, pterosin H (7), along with pterosin Z. A similar cyclization was also observed under base catalysis on alumina chromatography.[6] Such facile ring closure of hypacrone reflects the spatial proximity of both C atoms, C-5 and C-9, which implies the [Z]-configuration of the 6,7-double bond. In contrast to this, a geometrical isomer (9) of hypacrone, synthesized as described,[6] was unaffected by this treatment. This transformation suggests a biogenetic process in the plant for pterosins and hypacrone. On the chemical basis, the ring formation may be explained by a protonation–de-

†The stereochemistry is not obvious.

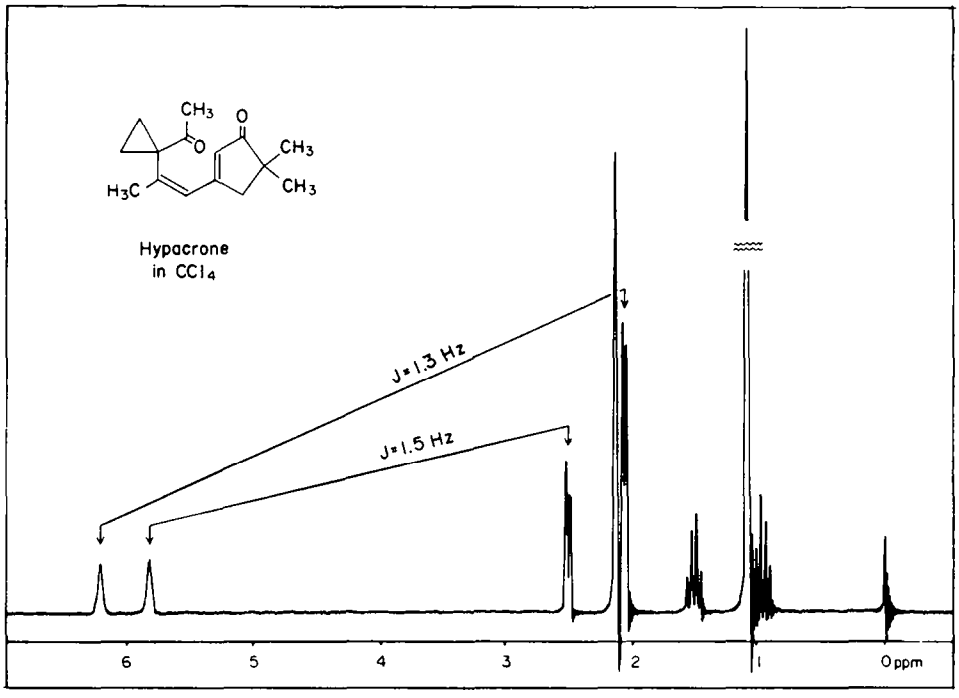
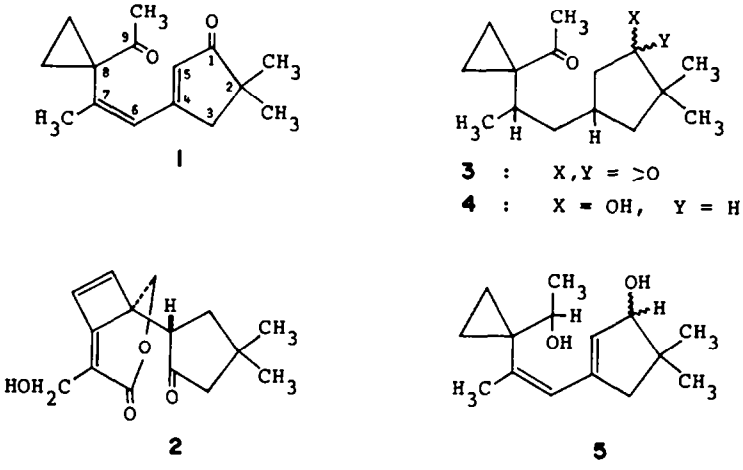


Fig. 1.



Scheme 1.

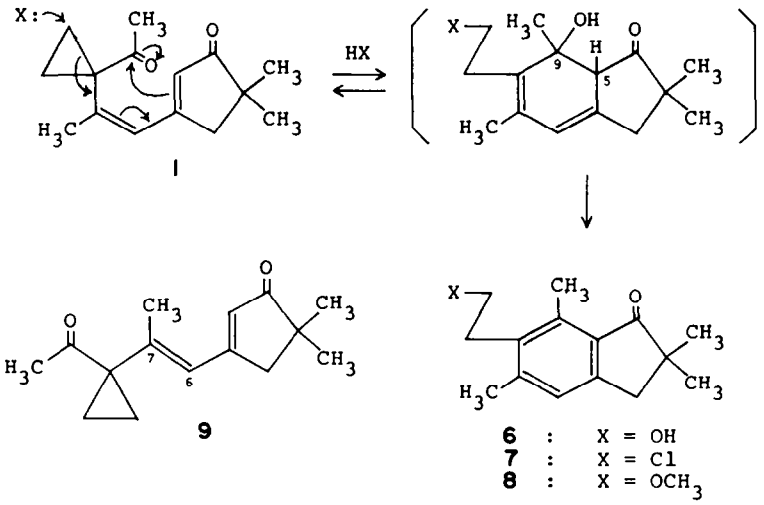
protonation sequence, including the elimination of one molecule of water, as shown in Scheme 2.

EXPERIMENTAL

IR spectra were recorded for liquid films or $CHCl_3$ solns on a Japan Spectroscopic Co. Model IR-E spectrometer, and UV spectra were measured in EtOH solns on a Hitachi Model 323 automatic recording spectrophotometer. NMR spectra were taken with a JEOL model PS-100 spectrometer (100 MHz) for chloroform-d or CCl_4 solns using TMS as an internal standard. Mass spectra were measured on a Hitachi Model RMU-6 or a JEOL Model JMS-01SG mass spectrometer. Analytical and preparative gas chromatographies (GLC) were performed by a Shimazu Model 4BMPF and a Varian Model 920 gas chromatograph, respectively, using 5% SE-30 packed column. All organic solvents were purified by distillation with glass joint apparatus before use.

Isolation of hypacrone (1)

Fresh shoots of *Hypolepis punctata* (10 Kg), collected in May–June at Nara Park, Nara, were extracted with 40 l. of boiling water for 30 min. The cooled and filtered aqueous soln was immediately passed through an active charcoal column (12 cm dia., 80 cm length, 1 kg of Wako active charcoal for chromatography). The column was washed thoroughly with water and then eluted with 20 l. of 95% EtOH and 20 l. benzene successively. The acrid substance was concentrated in the EtOH fraction. The EtOH was evaporated and the residual syrup (25 g) dissolved in 30 ml acetone. This soln was mixed with 20 g polyamide powder (Wako polyamide C-200 for chromatography) and evaporated to dryness by means of a rotary evaporator. The residual powder was placed on the top of a polyamide column (6 cm dia., 60 cm length, 100 g of Wako polyamide C-200), which was prepared with water as solvent. The column was eluted with water and the fractions were collected in each 15 ml portion. The acrid principle was found in fractions 66–145, which were combined and evaporated



Scheme 2.

in vacuo at room temp. After removal of some acetone insoluble material by filtration, the resulting pale yellow syrup (2.48 g) was finally purified by preparative layer chromatography (Merck Kieselgel GF_{254} Type 60, 20 × 20 cm, 1 mm thickness, 10 plates, chloroform:ether = 4:1 as solvent). The major band around R_f 6.0, which was easily detected by 2537 Å UV lamp, was collected and extracted with EtOAc to give 120 mg (0.0012%) of **1** as a colorless liquid with a very strong acrid taste, b.p. 90°/0.02 mm; MS (70 eV) *m/e* 232.1476 (M^+, calc. for $C_{15}H_{20}O_2$: 232.1463), 217.1237 (M^+–CH_3, calc. for $C_{14}H_{17}O_2$: 217.1228), 204.1170 (M^+–C_2H_4, calc for $C_{13}H_{16}O_2$: 204.1150), 189.1298 (M^+–CH_3CO, calc. for $C_{13}H_{17}O$: 189.1279); ν_{max}(neat) 1695, 1625, 1590 cm^{-1}; λ_{max} (EtOH) 285 nm (log ϵ: 4.26); δ (CCl_4) 0.95 (2H, m), 1.51 (2H, m), 1.06 (6H, s), 2.06 (3H, d, J = 1.3 Hz), 2.11 (3H, s), 2.49 (2H, d, J = 1.5 Hz), 5.78 (1H, br. s), 6.16 (1H, br.s).

Hydrogenation of hypacrone

(a) Hypacrone (15 mg) was hydrogenated with 10 mg of PtO_2 in 2 ml EtOAc for 2 hr under 1 atm of H_2. After filtration, the solvent was evaporated to give a syrup, which contained two major components on GLC analysis. Each component was separated by preparative layer chromatography on silica-gel. Tetrahydrohypacrone (**3**), 7 mg, MS (70 eV) *m/e* 236 (M^+); ν_{max}($CHCl_3$) 1730, 1680 cm^{-1}. Hexahydrohypacrone (**4**), 6 mg, MS (70 eV) *m/e* 238 (M^+); ν_{max}($CHCl_3$) 3300, 1685 cm^{-1}.

(b) A soln of **1** (18 mg) in 2 ml EtOH was treated with $NaBH_4$ (25 mg) at room temp for 17 hr. After working up as usual, the crude product was quickly purified by preparative layer chromatography (SiO_2) to give **5** as a colorless syrup, 14 mg, MS (70 eV) 236 (M^+); ν_{max}(neat) 2350 cm^{-1}.

Transformation of hypacrone into pterosins

A soln of **1** (19 mg) in 0.5 ml THF was warmed with 2 ml 10% H_2SO_4 at 90° for 3 hr. Extraction with ether and purification by preparative layer chromatography (SiO_2) gave pure crystals, 12 mg, m.p. 93°, which were identified with natural pterosin-Z by mixed m.p. and IR spectra. The treatment of **1** with 4N HCl in an analogous way (hypacrone 13 mg, 4N HCl 2 ml, AcOH 2 ml, refluxing for 4 hr) gave a mixture of pterosin-H (5 mg, m.p. 87°) and pterosin-Z (6 mg, m.p. 93°), both of which were separated by layer chromatography and identified with natural products.

Acknowledgements—Authors thank Dr. Y. Hirose of The Institute of the Food Chemistry, Osaka, and Japan Electron Optics Laboratory Co., Tokyo, for measurements of mass spectra, and Kasuga Shrine, Nara, for collection of the plant materials.

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