Studies on the Constituents of Medicinal and Related Plants in Sri Lanka. IV.\textsuperscript{1)} Isolation and Structure Determination of Hyperenone-B, Mysorenone-B, and Mysorenone-C from \textit{Hypericum mysorense} HEYNE and Synthesis of Hyperenone-A and -B

TOHRU KIKUCHI,* SHIGETOSHI KADOTA, SATOKO MATSUDA, and KEN TANAKA

Research Institute for Wakam-Yaku (Oriental Medicines),
Toyama Medical and Pharmaceutical University,
2630 Sugitani, Toyama 930–01, Japan

(Received August 24, 1984)

Three new compounds named hyperenone-B, mysorenone-B, and mysorenone-C were isolated from \textit{Hypericum mysorense} HEYNE, collected in Sri Lanka, and their structures were determined. In addition, hyperenone-A and -B, novel \(\gamma\)-pyrone derivatives, were synthesized.

Keywords—\textit{Hypericum mysorense}; Guttiferae; hyperenone-A; hyperenone-B; mysorenone-B; mysorenone-C; xanthone; \(\gamma\)-pyrone; synthesis

In a previous paper,\textsuperscript{2)} we reported the isolation and structure elucidation of several xanthone derivatives and three new compounds, hyperenone-A (1), mysorenone-A (2), and methyl phenacyl 1,1-dimethylprop-2-enylmalonate (3), from \textit{Hypericum mysorense} HEYNE (Guttiferae) collected in Sri Lanka. In a continuation of that work, we recently obtained three new compounds named hyperenone-B (4), mysorenone-B (12), and mysorenone-C (13) from the leaves of this plant. This paper describes the isolation and structure determination of these compounds and also a synthesis of hyperenone-A (1) and -B (4).

![Chart 1](image)

The ether extract of dried leaves of \textit{H. mysorense} was roughly separated by silica gel column chromatography and the fractions were further purified by preparative thin layer chromatography (TLC) to give hyperenone-B (4), mysorenone-B (12), and mysorenone-C (13) along with hyperenone-A (1), mysorenone-A (2), and known xanthone derivatives.

Hyperenone-B (4), mp 170—172 °C, showed the molecular ion peak at \(m/z\) 256 in the mass spectrum (MS) and its molecular formula was determined to be \(C_{16}H_{16}O_3\) by elementary analysis and high-resolution MS measurement. The ultraviolet (UV) spectrum of 4 exhibited maximum absorptions at 238 nm (log \(\varepsilon\) 4.19) and 329 nm (log \(\varepsilon\) 4.01), suggesting the presence of a conjugated aromatic chromophore. The proton nuclear magnetic resonance (\(^1\text{H}-\text{NMR})\) spectrum revealed signals due to an olefinic proton and two tertiary methyl groups, a vinyl moiety, and a mono-substituted phenyl group (see Experimental). This \(^1\text{H}-\text{NMR}\) pattern closely resembled that of hyperenone-A (1) except for the appearance of a hydroxyl signal at
δ 7.88 (disappeared on addition of deuterium oxide) instead of the methoxyl methyl signal in 1. In the MS, 4 showed strong fragment peaks at m/z 105 (C₉H₅CO⁺) and m/z 77 (C₆H₅⁺), indicating the presence of an oxygenic grouping at the benzylic position as in the case of 1.

From the above spectral data and the molecular formula, the structure of hyperenone-B was deduced to be 4. This was confirmed by the hydrolysis of hyperenone-A (1) under acidic conditions, giving a crystalline product (4), mp 169—171 °C, which was identical with natural hyperenone-B.

It should be noted here that the infrared (IR) spectrum of 4 in chloroform solution showed the carbonyl absorption at 1705 cm⁻¹, while in KBr disc the absorption appeared at 1650 cm⁻¹. The latter value is in accord with that of 1. On the other hand, the UV absorption of hyperenone-B (4) showed a remarkable bathochromic shift (329 nm) compared with that of hyperenone-A (1) (276 nm). Similar behavior was previously observed in the IR and UV spectra of 6-phenyl-2,4-pyronone (7) by Djerassi et al.,³ who reported that 7 appears to exist largely as the γ-pyrene tautomomer (8) in the solid state, but predominantly as the α-pyrene tautomomer (6) in ethanol solution. Likewise, hyperenone-B might be in the γ-pyrene form (4) in the crystalline state and in the α-pyrene form (5a) in solution.

Next, the synthesis of hyperenone-A (1) and -B (4) was undertaken for definite confirmation of their structures (Chart 2). The synthetic intermediate, 6-phenyl-2,4-pyronone (7), was prepared from ethyl benzoyleacetate by way of 11 according to Djerassi's description.³ This intermediate was converted to the sodium enolate and then treated with 3,3-dimethylallyl bromide in dimethylformamide to give the desired 4-O-dimethylallyl ether (9a) in 30—39% yield, along with O,C- and C,C-dialkylated products (9b and 10). Claisen rearrangement of 9a in dimethyl sulfoxide⁴ proceeded smoothly to afford a crystalline product (4), mp 170—172 °C, C₁₆H₁₆O₃, which was shown to be identical with authentic hyperenone-B (4) by mixed fusion and MS, IR (KBr), and ¹H-NMR comparisons.

Reaction of 4 with diazomethane in ether gave two products in a ratio of 7:3, and these were separated by preparative TLC. The major product showed mp 85—87 °C and was concluded to be the 4-O-methyl ether (5b) on the basis of its spectral data, while the minor product was found to be identical with natural hyperenone-A (1).

Mysorenone-B (12) is a minor component obtained as an oil. It showed the molecular ion
peak at \( m/z \) 230 in the MS and its molecular formula \( C_{15}H_{18}O_2 \) was established by high-resolution MS measurement. The UV spectrum of 12 exhibited maximum absorptions at 209, 252, and 312 nm (log \( \varepsilon \): 3.47, 3.41, and 3.61, respectively), corresponding to a conjugated aromatic chromophore. This UV pattern resembles that of myorenone-A (2). The \(^1\)H-NMR spectrum was closely similar to that of myorenene-A (2), but was characterized by the appearance of a singlet at \( \delta \) 2.40 assignable to an active methylene group and the absence of the methine and ester methyl protons in 2. In accordance with this finding, no ester carbonyl absorption was seen in the IR spectrum. Furthermore, the MS of 12 showed significant fragment peaks explainable as illustrated in Fig. 1.

In view of these spectral properties and the molecular formula, the structure of myorenene-B was deduced to be 12. This was confirmed by the following experiment. Alkaline hydrolysis of myorenene-A (2) with sodium hydroxide in aqueous methanol, followed by decarboxylation under acidic conditions, afforded solely an oily compound (12), which was found to be identical with natural myorenene-B. Thus the structure of myorenene-B was established to be 12.

![Diagram of chemical structures and reactions](image-url)
Mysorenone-C (13), mp 117—119 °C, is also a minor component of *H. mysorense*. It showed the molecular ion peak at *m/z* 244 in the MS and its molecular formula was determined to be C_{15}H_{16}O_{3} by high-resolution MS. The UV spectrum of 13 gave absorptions at 247 and 314 nm, similar to those of mysorenone-B (12), and the ^1^H-NMR spectrum showed characteristic signals arising from an olefinic proton and a mono-substituted phenyl, a vinyl, and two tertiary methyl groups (see Experimental), suggesting that this compound (13) is closely related to mysorenone-B (12).

On the basis of the above spectral data and the biogenetic analogy, together with the MS fragmentation pattern, which could be interpreted as shown in Fig. 1, the structure of mysorenone-C should be represented by the formula 13.

Interestingly, the hydroxyl proton signal in the ^1^H-NMR spectrum of 13 was markedly shifted upfield (δ 3.79) compared with that of 12 (δ 16.15) and the IR spectrum in chloroform solution exhibited a hydroxyl band at 3300 cm^{-1} and a carbonyl absorption at 1700 cm^{-1} corresponding to a conjugated five-membered ketone. Therefore, mysorenone-C appears to exist predominantly as the cyclic hemiketal form (14) in chloroform solution, but as the tautomer 13 in ethanolic solution. Support for the possibility of the structure 14 was provided by the MS fragment peak observed at *m/z* 102 (C_{6}H_{5}C≡CH ^{+}), which may be explained by the fragmentation at the dihydrofuranone ring (Fig. 1). Further, it is interesting to note that the IR spectrum of mysorenone-C in potassium bromide pellet showed the hydroxyl absorption at 3150 cm^{-1} (broad) and the carbonyl absorption at 1665 cm^{-1}. Thus, it is possible that the alternative tautomeric form 15 contributes to the structure of mysorenone-C in the solid state, although no definitive evidence is available.

**Experimental**

Melting points were determined on a Kofler-type apparatus and are uncorrected. UV spectra were taken in EtOH solutions and IR spectra in CHCl_{3} solutions, unless otherwise noted. ^1^H-NMR spectra were measured on a Varian XL-200 spectrometer in CDCl_{3} solutions using tetramethylsilane as an internal standard and chemical shifts are recorded in δ values. MS and high-resolution MS were obtained with a JEOL JMS-D 300 spectrometer (ionization voltage, 70eV; accelerating voltage, 30eV) using a direct inlet system and relative intensities of peaks are given in parentheses. Gas chromatography (GC) was done on a Shimadzu GC-6AM instrument with a 2% OV-17 column (2 m × 3 mm i.d. glass tube; injection temp., 230—260 °C; column temp., 220—230 °C; carrier gas, nitrogen). Column chromatography was carried out with Mallinckrodt silica gel, and the eluted solutions were concentrated *in vacuo*. For TLC, Merck Kieselgel 60 F_{254} was used and spots were detected by coloration with Ce(SO_{4})_{2}-H_{2}SO_{4} reagent or under UV light. For preparative TLC, Merck Kieselgel PF_{254} or F_{254} (0.5 mm thickness) was employed and plates were observed under UV light. Extraction of substances from silica gel was done with MeOH–CH_{2}Cl_{2} mixture (5:95 or 1:9) and solutions were concentrated *in vacuo*.

**Extraction, Isolation, and Properties of New Compounds from *H. mysorense*** — Air-dried leaves (1 kg) of *H. mysorense*, collected at Horton Plains, Sri Lanka, in the flowering season (July, 1983), were extracted with ether (5 *l* × 3) for 7 d at room temperature and the combined solutions were concentrated to give an ether extract (34 g). The plant material was then extracted with boiling MeOH (5 l × 3) for 3 h to give a methanol extract (550 g).

The ether extract was subjected to silica gel (500 g) column chromatography, eluted successively with CH_{2}Cl_{2} (F-1, 22.5 g), MeOH–CH_{2}Cl_{2} (1:99) (F-2, 2.7 g), MeOH–CH_{2}Cl_{2} (3:97) (F-3, 0.6 g), MeOH–CH_{2}Cl_{2} (5:95) (F-4, 5.4 g), and MeOH–CH_{2}Cl_{2} (15:85) (F-5, 0.5 g). F-2 was again chromatographed on silica gel (60 g) and the CH_{2}Cl_{2} eluate (0.4 g) was further purified by preparative TLC using CHCl_{3} as the eluent, whereupon mysorenone-A (2) (25 mg), an oil, was obtained from the less polar fraction and hyperenone-B (4) (200 mg) from the more polar fraction.

F-3 was also separated into two fractions by preparative TLC using MeOH–CHCl_{3} (2:98) as the developing solvent. The less polar fraction, on trituration with ether–hexane, gave hyperenone-A (1) (135 mg), colorless prisms, mp 81—82 °C. The mother liquor (300 mg) was again separated by preparative TLC with CHCl_{3} as the developing solvent, giving mysorenone-B (12) (8 mg) as the more mobile fraction and an additional crop of mysorenone-A (2) (9 mg) as the less mobile fraction.

The more polar fraction was a crystalline mass, which was recrystallized from ether–hexane to afford 1,7-dihydroxyxanthone (80 mg), yellow needles, mp 239—242 °C. The mother liquor (120 mg) was further purified by repeated preparative TLC to give mysorenone-C (13) (6 mg).

Hyperenone-B (4): Colorless prisms (from ether–hexane), mp 170—172 °C. UV λ _{max}, nm (log ε): 205 (4.30), 238...
(4.19), 329 (4.01). IR νmax cm⁻¹: 3400, 1705, 1650, 1560 (in CHCl₃); 3400, 1650, 1620, 1650 (in KBr). ¹H-NMR δ: 1.56 (3H × 2, s, tert-CH₃), 5.48, 5.58 (each 1H, dd, J=1, 11 and 1, 17 Hz, respectively; =CH₂), 6.32 (1H, s, =CH), 6.46 (1H, dd, J=11, 17 Hz; =CH), 7.48 (3H, m, aromatic protons), 7.80 (2H, m, aromatic protons), 7.88 (1H, s, OH; disappeared on addition of D₂O). MS m/z (rel. int.): 256 (M⁺, 60), 241 (M⁺−CH₂Cl, 55), 201 (50), 188 (McLafferty fragment, 45), 105 (100), 77 (40), 69 (45). High-resolution MS: Found: 256.053, Calculated for C₄H₆O₂ (M⁺) 256.0599; Found: 241.0853, Calculated for C₁₂H₁₄O₂ 241.0864; Found: 201.0509, Calculated for C₁₂H₁₀O₂ 201.0552; Found: 188.0459, Calculated for C₁₁H₁₀O₂ 188.0473; Found: 105.0339, Calculated for C₄H₄O 105.0340; Found: 77.0375, Calculated for C₃H₄ 77.0391; Found: 69.0690, Calculated for C₃H₄ 69.0704. *Anal. Caled for C₁₂H₆O₂: C, 74.98; H, 6.29. Found: C, 74.68; H, 6.24.*

Mysorenone-B (12): Slightly colored oil. UV λmax nm (log ε): 209 (3.47), 252 (3.41), 312 (3.61). IR νmax cm⁻¹: 1605 (br), 1575, 1465. ¹H-NMR δ: 1.18, 1.27 (each 3H, s, tert-CH₃), 2.40 (2H, s, CO–CH₂–), 5.00, 5.015 (each 1H, d, J=11 and 17 Hz, respectively; =CH₂), 5.97 (1H, dd, J=11, 17 Hz; =CH), 6.14 (1H, s, =CH), 7.51 (3H, m, aromatic protons), 7.80 (2H, m, aromatic protons), 16.15 (1H, s, C=O–OH; disappeared on addition of D₂O). MS: See Fig. 1. High-resolution MS: Found: 230.1312, Calculated for C₁₁H₁₄O₂ (M⁺) 230.1307; Found: 215.1072, Calculated for C₁₀H₁₂O₂ 215.1072; Found: 162.0686, Calculated for C₁₀H₁₀O₂ 162.0681; Found: 161.0555, Calculated for C₁₁H₁₀O₂ 161.0602; Found: 147.0437, Calculated for C₁₀H₈O₂ 147.0446; Found: 105.0342, Calculated for C₄H₈O 105.0340; Found: 77.0389, Calculated for C₃H₄ 77.0391; Found: 69.0689, Calculated for C₃H₄ 69.0704.

Acid Hydrolysis of Hypernone-A (1) — Ten percent HCl (0.5 ml) was added to a solution of hypernone-A (1) (5 mg) in acetone (0.5 ml) and the mixture was stirred at room temperature overnight. The mixture was then subjected to preparative TLC with acetone–CHCl₃ (2:98) as the eluent to give a crystalline product (2) (2 mg), which on recrystallization from ether–hexane gave colorless prisms, mp 169–171 °C. This product was found to be identical with authentic hypernone-B (4) by GC, MS, IR, and ¹H-NMR comparisons. A small amount of the starting material (1) (1.3 mg) was recovered from the more polar fraction.

Synthesis of Hypernone-A (1) and -B (4) — 1.) 3,3-Dimethylallylation of 6-Phenyl-2,4-pyronone (7): A solution of 7 (200 mg) in MeOH (10 ml) was mixed with 0.1 N NaOH–MeOH (10 ml), and the solution was concentrated to dryness in vacuo. The residue was dissolved in N,N-dimethylformamide (DMF) (10 ml) and then a solution of 3,3-dimethylallyl bromide³⁰ (206 mg) in DMF (10 ml) was added dropwise with vigorous stirring at room temperature. Stirring was continued for 3 h, then the reaction mixture was diluted with ether (50 ml) and washed with water (30 ml × 3). The water layer was extracted with ether (30 ml × 2). The combined ether solutions were dried (anhyd. MgSO₄) and concentrated to give a crystalline residue, which was subjected to preparative TLC with CHCl₃ as the developing solvent. The least polar fraction (58 mg) gave the C,C-dialkyl product (10), oil. UV λmax nm (log ε): 223 (4.03), 228 (4.02), 303 (4.23). IR νmax cm⁻¹: 1780, 1665, 1630. ¹H-NMR δ: 1.63 (3H × 4, br, s, vinyl CH₂), 2.75 (2H × 2, d, J=7.5 Hz, C₂–CH₂–CH), 5.05 (1H × 2, t, J=7.5 Hz, C₂–CH₂–CH), 6.35 (1H, s, C=CH), 7.4–8.2 (5H, aromatic protons). The next fraction (25 mg) afforded the O,C-dialkylated product (9b), colorless prisms (from ether–hexane, mp 91–92 °C). UV λmax nm (log ε): 241 (4.28), 337 (4.16). IR νmax cm⁻¹: 1700, 1640, 1500. ¹H-NMR δ: 1.78 (3H × 4, s, vinyl CH₂), 3.20 (2H, d, J=7 Hz, =C–CH₂–CH), 4.72 (2H, d, J=6 Hz, O–CH₂–CH), 5.26 (1H, t, J=7 Hz, =C–CH₂–CH), 3.57 (1H, t, J=6 Hz, O–CH₂–CH), 6.63 (1H, s, =CH), 7.47 (3H, m, aromatic protons), 7.83 (2H, m, aromatic protons). *Anal. Caled for C₁₂H₁₀O₂: C, 77.75; H, 7.46. Found: C, 78.25; H, 7.43. The third fraction (75 mg) gave 4-(3,3-Dimethylallyloxy)-6-phenyl-s-pyronone (9a), colorless needles (from ether–hexane), mp 65–69 °C. UV λmax nm (log ε): 426 (4.09), 255 (4.10), 317 (3.99). IR νmax cm⁻¹: 1720, 1640, 1500. ¹H-NMR δ: 1.76, 1.83 (each 3H, s, vinyl CH₂), 4.56 (2H, d, J=6 Hz, O–CH₂–CH), 5.46 (1H, t, J=6 Hz, O–CH₂–CH), 5.54, 6.46 (each 1H, d, J=2 Hz, =CH), 7.50 (3H, m, aromatic protons), 7.83 (2H, m, aromatic protons). MS m/z (rel. int.): 256 (M⁺, 61), 241 (100), 213 (27), 105 (56), 77 (32). *Anal. Caled for C₁₆H₁₃O₂: C, 74.98; H, 6.29. Found: C, 74.74; H, 6.39. From the starting line unchanged, the uncharged starting material (7) (45 mg) was recovered.

2) Clausen Rearrangement of 4-(3,3-Dimethylallyloxy)-6-phenyl-s-pyronone (9a): A solution of 9a (50 mg) in DMSO (10 ml) was heated for 3 h at 130 °C in an oil bath. After evaporation of DMSO in vacuo, the residue was purified by preparative TLC (developed with CHCl₃) and then recrystallized from ether–hexane to give colorless prisms (4) (45 mg), mp 170–172 °C. *Anal. Caled for C₁₆H₁₄O₂: C, 74.98; H, 6.29. Found: C, 75.22; H, 6.22. This product was shown to be identical with hypernone-B (4) by mixed fusion and GC, MS, IR, and ¹H-NMR comparisons.

3) Methylation of 4 with Diazomethane: Crystals of 4 (20 mg) were added to a solution of excess CH₃I in ether (8 ml) and the mixture was left at room temperature overnight. The reaction mixture showed two peaks on GC in a ratio of 7:3. After evaporation of the solvent, the residue was separated by preparative TLC with MeOH–CHCl₃.
(1:99) as the eluent. The more mobile fraction gave the 4-methyl ether (5b) (14 mg), colorless prisms (from ether–hexane), mp 85–87°C. IR \( \nu_{\text{max}} \text{ cm}^{-1} \): 1700, 1640, 1530. \(^1\)H-NMR \( \delta \): 1.50 (3H × 2, s, \text{teta-CH}_3\), 3.90 (3H, s, OCH\(_3\)), 4.89, 4.96 (each 1H, dd, \( J = 1, 11 \text{ Hz and 1, 18 Hz, respectively} \)), 6.22 (1H, dd, \( J = 11, 18 \text{ Hz, CHJ-CH}_3\)), 6.55 (1H, s, =CH), 7.47 (3H, m, aromatic protons), 7.84 (2H, m, aromatic protons). MS \( m/z \): 270 (M\(^+\), 45), 255 (60), 227 (80), 215 (70), 125 (10), 105 (10), 77 (45). \textit{Anal.} Caled for C\(_{17}\)H\(_{16}\)O\(_2\): C, 75.53; H, 6.85. Found: C, 75.60; H, 6.70. The less mobile fraction gave the 2-methyl ether (1) (6 mg), colorless prisms (from ether–hexane), mp 81–82°C. \textit{Anal.} Caled for C\(_{17}\)H\(_{16}\)O\(_2\): C, 75.53; H, 6.85. Found: C, 75.81; H, 6.70. This product was shown to be identical with hyperenone-A (1) by mixed fusion and GC, IR, MS, and \(^1\)H-NMR comparisons.

**Hydrolysis and Decarboxylation of Mysorenone-A (2)**—A mixture of mysorenone-A (2) (4 mg), 2% aq. NaOH (0.6 ml), and MeOH (2.2 ml) was heated for 1 h in a water bath (bath temperature, 80°C), then the mixture was acidified by addition of 10% HCl (0.8 ml) and heated for 1.5 h at 80°C. After evaporation of the solvent \textit{in vacuo}, the residue was taken in ether, and the ether solution was washed with brine, dried (anhyd. MgSO\(_4\)), and concentrated. The residue was purified by preparative TLC with CHCl\(_3\) as the eluent to give an oily product (12) (3.5 mg). This was shown to be identical with mysorenone-B (12) by GC, GC-MS, and \(^1\)H-NMR comparisons.

**Acknowledgment** This work was supported in part by a Grant-in-Aid for Overseas Scientific Survey from the Ministry of Education, Science and Culture, and also by Grants from the Division of Drug Industry of Toyama Prefecture and the Pharmaceutical Industry Division of Toyama City, which are gratefully acknowledged. Thanks are also due to Mr. M. Ogawa of this University for microanalyses.

**References and Notes**


5) Mysorenone-C (13) is rather unstable and it decomposes to a complex mixture on standing in solution in EtOH or CDCl\(_3\), for 3–7 d.