The Superoxide Release Inhibitors, Cryptoporic Acids C, D, and E; Dimeric Drimane Sesquiterpenoid Ethers of Isocitric Acid from the Fungus *Cryptoporus volvatus*

Toshihiro Hashimoto,[a] Motoo Tori,[a] Yasuo Mizuno,[a] Yoshinori Asakawa,*[a] and Yoshimasa Fukazawa[b]

[a] *Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro cho, Tokushima 770, Japan*
[b] *Department of Chemistry, Faculty of Science, Hiroshima University, Higashisenda-machi, Naka-ku, Hiroshima 730, Japan*

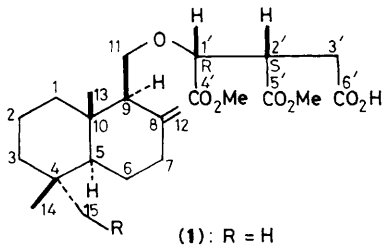
Three novel dimeric drimane sesquiterpenoid ethers of isocitric acid, cryptoporic acids C, D, and E, which are bitter principles and superoxide release inhibitors, have been isolated from the fungus *Cryptoporus volvatus* and the structures determined by spectroscopic methods, chemical transformations, and X-ray analysis.

Recently, we reported the isolation and structure determination of two novel bitter principles, cryptoporic acids A (**1**) and B (**2**), which were drimane sesquiterpenoid ethers of albicanol (**3**) linked by an ether bond to isocitic acid dimethyl ester, isolated from the fungus *C. volvatus* (Polyporaceae).[1] This paper deals with the isolation of three novel bitter dimeric drimane sesquiterpenoids, cryptoporic acids C (**4**), D (**5**), and E (**6**) having superoxide release inhibitory activities.

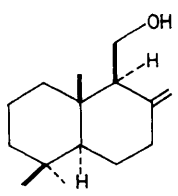
Ethyl acetate extract (75.6 g) of fresh *C. volvatus* (1.6 kg) was subjected to a combination of Sephadex LH-20 ($CHCl_3$–EtOH; 1 : 1) and silica gel ($CHCl_3$–EtOH) column chromatography to afford (**4**) (5.0 g), (**5**) (5.8 g) and (**6**) (6.0 g).†

† Compounds show satisfactory spectral data including high resolution mass spectra.

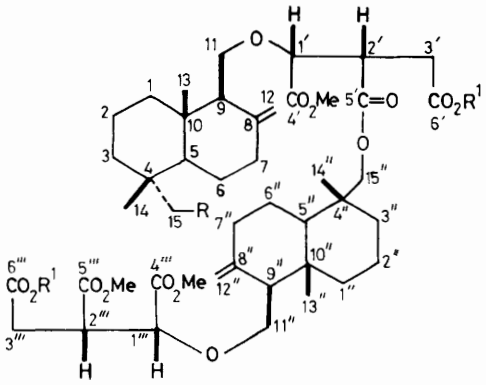
Cryptoporic acid C (**4**), $[\alpha]_D$ +61.2°, $C_{45}H_{68}O_{14}$, indicated the presence of two carboxy (3400—2400 and 1715 cm^{-1}; δ_C 178.2 and 178.7) and three methoxycarbonyl (1740 and 1730 cm^{-1}; δ 3.69, 3.76, and 3.77) groups. The ^1H and ^{13}C n.m.r. spectra



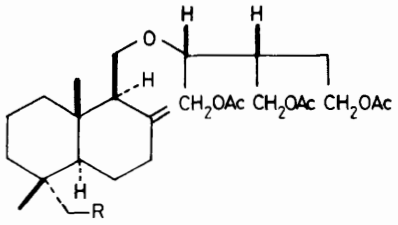
(**1**): R = H
(**2**): R = OH



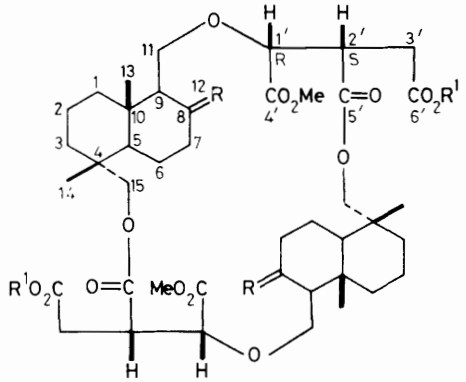
(**3**)



(4): R = R[1] = H
(6): R = OH, R[1] = H
(7): R = H, R[1] = Me
(12): R = OH, R[1] = Me



(8): R = H
(9): R = OAc



(5): R = CH_2, R[1] = H
(10): R = CH_2, R[1] = Me
(11): R = O, R[1] = Me

of pentamethyl ester (7)† obtained by treatment of (4) with diazomethane confirmed the presence of five methoxycarbonyl groups. Lithium aluminium hydride reduction of (7) followed by acetylation afforded (8) and (9)† which were identical with the compounds derived from (1) and (2), respectively. Thus the gross structure of (7) was deduced as depicted. The positions of the two carboxy groups and the ester linkage between the two sesquiterpene units were determined to be 6′ and 6‴, and 5′, respectively, as long-range couplings (between 6′–CO and H–3′, 6‴–CO and H–3‴, 5′–CO and H–2′) were detected in the long-range ^{13}C–^{1}H correlation spectrum.

As the ^{1}H n.m.r. spectrum of cryptoporic acid (5) was simple and only half the carbon signals appeared, (5) was deduced to be a symmetric dimer. Its tetramethyl ester (10),† m.p. 238—241 °C (decomp.), $[\alpha]_D$ +30.9° ($CHCl_3$), $C_{44}H_{64}O_{14}$, was converted to (9) (using $LiAlH_4$ and then Ac_2O). *X*-Ray crystallographic analysis of diketone (11)†‡ [ozonolysis of (10) followed by Zn/AcOH] was carried out to establish the stereochemistry. The absolute configuration was determined by the c.d. spectrum of (11), which showed a negative Cotton effect at λ_{max} 276 nm [(θ) − 1876].

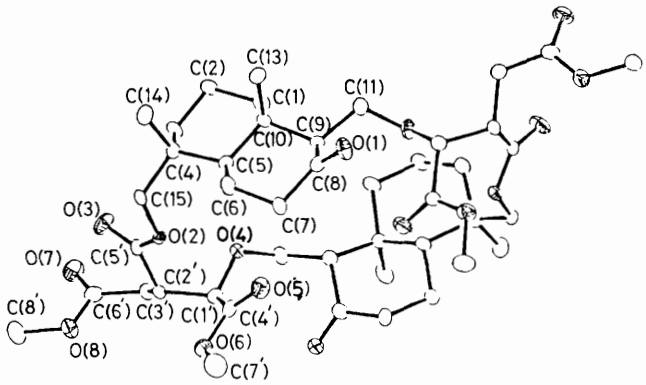


Figure 1. Crystal structure of (11).

The ^{1}H and ^{13}C n.m.r. spectra of cryptoporic acid E (6), $[\alpha]_D$ +42.6° ($CHCl_3$), $C_{45}H_{68}O_{15}$, suggested that this compound was a dimer of (2). Pentamethyl ester (12) was treated in the same manner as described above to give (9). The long-range ^{13}C–^{1}H correlation spectrum of (6) revealed the position of the carboxy and the ester groups as depicted.

It is interesting that the three new compounds (4), (5), and (6) have a persistent bitter taste. Cryptoporic acids C (4)§ and E (6) inhibited the release of superoxide anions[2] from guinea-pig peritoneal macrophage induced by O_2^- stimulant FMLP (formyl methionyl luecyl phenylalanine; 10^{-7} M) at I.C.$_{50}$ 0.07 and 0.05 μg/ml, respectively.

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References

1 T. Hashimoto, M. Tori, Y. Mizuno, and Y. Asakawa, *Tetrahedron Lett.*, 1987, 6303.
2 T. Matsumoto, K. Takeshige, and S. Minakami, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 974.

‡ *Crystal data*: (11), $C_{44}H_{64}O_{16}$, M_r = 848.95, monoclinic, space group *C*2, *a* = 24.092(9), *b* = 8.337(2), *c* = 11.047(4) Å, β = 101.16(3)°, V = 2176.9 (1.3) Å^3, *Z* = 2, D_c = 1.30 g/cm^3. *X*-Ray data were collected by the use of graphite-monochromated Mo-K_α radiation (λ = 0.71073 Å) on a Syntex R3 automatic four-circle diffractometer to 2θ = 55.0°. Of 2664 total unique reflections, 1870 were considered to be observed $F_o > 3.0\sigma(F_o)$. Data were corrected for Lorentz and polarization effect, but not for absorption (μ = 1.1 cm^{-1}). The structure was solved by direct methods (MULTAN78). All nonhydrogen atoms were located in the initial E synthesis. Hydrogen atoms were included in calculated positions (C–H 1.08 Å). Block-diagonal least-squares refinement with 30 nonhydrogen atoms anisotropic and 32 hydrogens isotropic converged to a conventional *R* factor of 0.089. All the calculation were done on a HITAC M-200H computer of the Hiroshima University using the structure analysis program system UNICS3 (T. Sakurai and K. Kobayashi, *Rep. Inst. Phys. Chem. Res.*, 1979, **56**, 69). Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

§ Cryptoporic acid C (4) also inhibited the release of O_2^- from rabbit polymorphonuclear leucocyte induced by O_2^- stimulant FMLP (10^{-7} M) at I.C.$_{50}$ 2 μ g/ml.