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STRUCTURAL AND CONFORMATIONAL STUDIES ON EUPHOHELIOSCOPINS A AND B AND RELATED DITERPENES

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Summary: Two new toxic diterpenes (euphohelioscopins A and B) and two euphoscopin-type ones (euphoscopins C and D) have been isolated from the plant Euphorbia helioscopia L., and their stereostructures and conformations also been elucidated on the basis of their spectral data and some chemical evidence, together with successful application of molecular mechanics calculations to such flexible molecules as described herein.

In connection with polyoxygenated diterpenes which have antitumor activity or promote cancer development in tumor formation, euphoscopins A and B have been isolated from the plant Euphorbia helioscopia L.[1] Further investigation of toxic substances in the same plant resulted in the isolation of two new different type of diterpenes (euphohelioscopins A and B) and two new euphoscopin-type ones (euphoscopins C and D) in addition to euphoscopins A and B.

As described in the previous paper,[2] the MeOH extract of the leaves and roots of the same plant (50 Kg) was washed with isooctane, and then partitioned between ether and water. The ethereal extract was roughly separated by column chromatography on silica gel (Mallinckrodt, 100 mesh) using a gradient solution of hexane - AcOEt, and then further separated by repeating preparative TLC (Kieselgel PF_{254}) using AcOEt - hexane (1 : 1~3), AcOEt - benzene (1 : 3~7) and/or AcOEt - CH_2Cl_2 (1 : 20 or 30) to afford euphohelioscopins A and B (1 and 2) in addition to euphoscopins A, B, C and D (3~6) [1, 195 mg; 2, 4.1 mg; 3, 174 mg; 4, 331 mg; 5, 16 mg; 6, 21 mg].[3] The physical data of euphohelioscopins A and B are shown below.

Euphohelioscopin A (1) as a colorless oil: $C_{30}H_{42}O_6$ [m/e 498.2986(M^+)]; IR (film) 3500, 1735, 1710, 1620br.cm^{-1}; $\delta(C_6D_6)$ 0.72(3H, t, J= 6.5Hz), 0.85(3H, s), 0.96(3H, s), 1.00(3H, d, J= 6Hz), 1.58(3H, br.s), 1.65(3H, s), 1.93(3H, s), 2.61(1H, dd, J= 7, 11Hz), 2.93(1H, dd, J= 7.5, 14Hz), 3.57(1H, dd, J= 4, 7Hz), 5.00(1H, dd, J= 3, 10.5Hz), 5.56(1H, dt, J= 11, 7.5Hz), 5.98(1H, d, J= 15Hz), 6.00(1H, br.t, J= 11Hz), 6.31(1H, br.d, J= 11Hz), 6.62(1H, br.d, J= 11Hz), 7.87(1H, dd, J= 11, 15Hz).

Euphohelioscopin B (2) as a colorless oil: $C_{30}H_{42}O_7$ [m/e 514.2964(M^+)]; IR (film) 3500, 1720 br., 1645, 1615cm^{-1}; $\delta(CDCl_3)$ 0.96(3H, t, J= 6.5Hz), 1.07(3H, d, J= 6Hz), 1.08(3H, s), 1.18(3H, s), 1.55(3H, br.s), 1.83(3H, s), 2.03(3H, s), 2.59(1H, dd, J= 6, 11Hz), 2.89(1H, dt, J= 2, 5.5Hz), 3.17(1H, dd, J= 2, 7Hz), 3.83(1H, dd, J= 3, 6.5Hz), 4.90(1H, dd, J= 3, 10.5Hz), 6.04(1H, br.d, J= 11Hz), 6.12(1H, d, J= 15.5Hz), 6.56(1H, br.d, J= 11Hz), 6.71 (1H, dd, J= 7, 15.5Hz).

As judged from their ^1H NMR spectra, probably, euphohelioscopins A and B both have the

same carbon skeleton. In fact, on treatment with K_2CO_3 - MeOH (room temp., 18 h), both 1 and 2 were converted into the same triol [7: 139 - 140.5 °C; $C_{20}H_{30}O_4$(m/e 334.2128(M^+))], in high yield. In the case of 1, furthermore, an $\alpha\beta,\gamma\delta$-unsaturated ester (8) was obtained, whose structure was based on J-value of the olefinic protons (δ5.84, 5.87, 6.14 and 7.64).[4] Although the corresponding methyl ester has not been isolated in the case of 2, euphohelioscopin B (2) should have an $\alpha\beta$-unsaturated ester group containing an epoxide ring at C_4-C_5 position, on the basis of a comparison of ^{1}H NMR spectra between 1 and 2 (δ5.56, 5.98, 6.00 and 7.87 in 1; δ2.89, 3.17, 6.12 and 6.71 in 2). Particularly, the geometry of the epoxide ring must be trans, as judged from the coupling constant (J= 2Hz) between C_4-H and C_5-H,[5] while the corresponding J-value is 4.5Hz in the cis epoxide (9)[6] produced on treatment of 8 with m-chloroperbenzoic acid.

On acetylation with Ac_2O (2 eq) - pyridine (room temp., 10 h), the triol (7) was readily converted into a diacetate (10) in addition to two monoacetates (11 and 12).[7] The diacetate (10) has the following physical data: mp 153 - 154 °C; $C_{24}H_{34}O_6$(m/e 418.2371(M^+); IR (film) 3460, 1735, 1720sh., 1640sh., 1610br.cm^{-1}; δ(C_6D_6) 0.55 - 0.9(1H, m)(C_9-H), 0.97(6H, s)(C_{10}-Me), 1.03(3H, d, J= 6.0Hz)(C_2-Me), 1.18(1H, dd, J= 7.8, 11.4Hz)(C_{11}-H), 1.53(3H, d, J= 1.4Hz)(C_6-Me), 1.74(3H, s)(OAc), 1.80(3H, s)(OAc), 1.84(3H, d, J= 1.2Hz)(C_{13}-Me), 1.4 - 2.6(5H, complex)(C_1-H, C_2-H and C_8-H), 2.78(1H, dd, J= 8.4, 11.1Hz)(C_4-H), 3.03(1H, br.s)(OH), 4.80 (1H, dd, J= 6.0, 8.4Hz)(C_3-H), 4.86(1H, dd, J= 3.1, 11.3Hz)(C_7-H), 5.92(1H, dd, J= 1.4, 11.1 Hz)(C_5-H), 7.29(1H, dd, J= 1.2, 11.4Hz)(C_{12}-H). On the basis of the ^{1}H NMR spectrum with aid of decoupling experiments, a partial structure [A] must be present in 10. Furthermore, in the light of co-occurrence of euphoscopins A and B (3 and 4),[1] euphohelioscopin A must have structure (1), in which the partial structure [A] is accommodated, except for the stereochemistry and positions of the two ester groups.

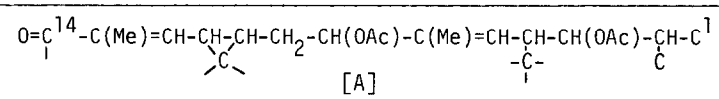
Oxidation of 1 with PCC in CH_2Cl_2 (room temp., 10 h) afforded a conjugated dione (13) which has neither AcO group nor C_4-H proton,[8] indicating that the $\alpha\beta,\gamma\delta$-unsaturated ester group and the AcO group must be located at C_7- and C_{15}-positions, respectively.

The stereochemistry of 1 was elucidated by NOE experiments in the ^{1}H NMR spectrum of 10 (see [B]) together with some chemical evidence, as follows. When treated with PhCOCl - pyridine (room temp., 9 h), the monoacetates (11 and 12) were converted into the corresponding benzoates (14 and 15), respectively [14: $C_{29}H_{36}O_6$(m/e 480.2524(M^+)); δ($CDCl_3$) 1.28(3H, s)(C_7-OAc) and 5.02(1H, dd, J= 6, 8.5Hz)(C_3-H). 15: $C_{29}H_{36}O_6$(m/e 480.2538(M^+)); δ($CDCl_3$) 1.56 (3H, s)(C_3-OAc) and 5.06(1H, dd, J= 3, 11Hz)(C_7-H)]. As seen in the case of euphoscopin B (4), the NMR signal due to the AcO group at C_7-position in 14 was observed in higher magnetic field (δ1.28) rather than that in 11 (δ2.01), indicating that the two ester groups must be present in the same side. Furthermore, the configuration at C_{15}-position was based on the following chemical evidence: on treatment with 1,1-carbonyldiimidazol (room temp., 1.5 h and then 140 °C, 3 days), 11 was successfully converted into a carbonate ester [16: $C_{23}H_{30}O_6$(m/e 402.2043(M^+)); IR (film) 1770 and 1735cm^{-1}], indicating that both AcO and OH groups in 1 are also present in the same side. Finally, the stereochemistry at C_9- and C_{11}-positions and conformation of 1 were determined by means of molecular mechanics calculations[9] of the diacetate (10), in which the four possible geometrical isomers at C_9- and C_{11}-positions are considered. The most stable conformers (I~IV) in each isomer were selected by means of molecular mechanics calculations. In each case, the steric energy of the most stable conformer was

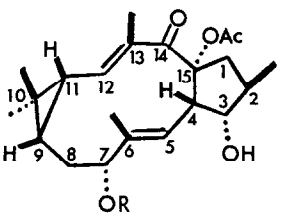
quite small as compared with those of the other possible ones.[10] Finally, the coupling constants of each proton were calculated on the basis of the most stable conformation, and then compared with the observed ones. Thus, the conformation [I] was only compatible with the observed data (see Table 1). Euphohelioscopins A and B belong to a group of lathylane-type diterpenes.[11]

Table 1

Proton	J (Calcd) (Hz)	J (Found) (Hz)	Proton	J (Calcd) (Hz)	J (Found) (Hz)
C_2-H, C_3-H	5.9	6.0	C_7-H, C_8-H'	4.2	3.1
C_3-H, C_4-H	7.9	8.4	C_9-H, C_{11}-H	9.8	7.8
C_4-H, C_5-H	13.0	11.1	C_{11}-H, C_{12}-H	9.1	11.4
C_7-H, C_8-H	12.8	11.3			

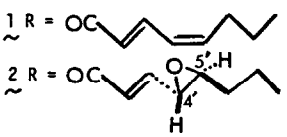


[A]



1 R = OC

2 R = OC



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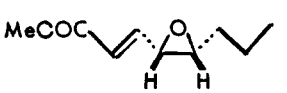

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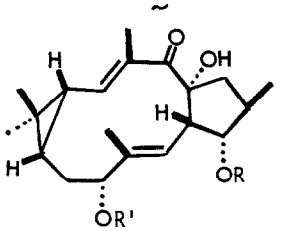
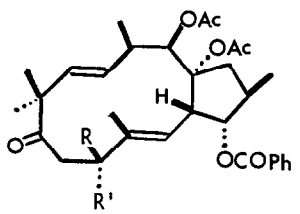
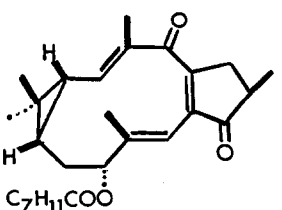


7 R = R' = H
10 R = R' = Ac
11 R = H, R' = Ac
12 R = Ac, R' = H
14 R = PhCO, R' = Ac
15 R = Ac, R' = PhCO



3 R = H, R' = OH
4 R = H, R' = OAc
5 R = H, R' = OCOPh
6 R,R' = O



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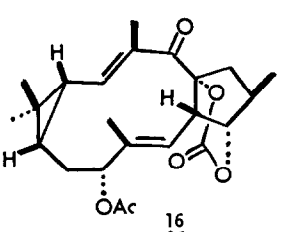

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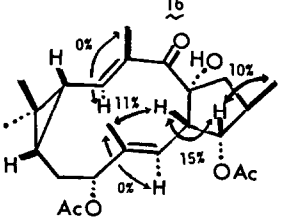
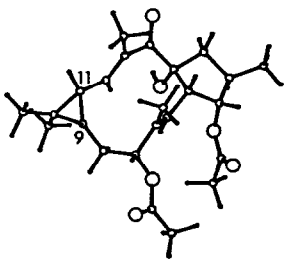
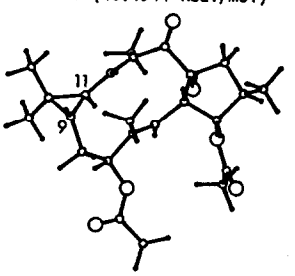


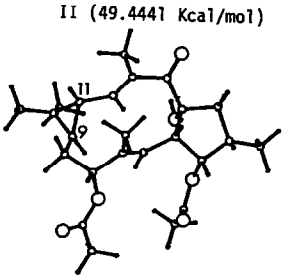
[B]



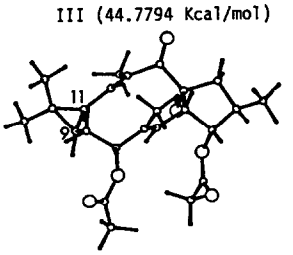
I (40.4944 Kcal/mol)



II (49.4441 Kcal/mol)



III (44.7794 Kcal/mol)



IV (50.5174 Kcal/mol)

The spectral data of euphoscopins A~D (3~6),[12] of which the structures of euphoscopins A and B (3 and 4) have been elucidated by means of an X-ray crystallographic analysis,[1] are quite similar to one another, particularly in their ^{1}H NMR spectra.

Euphoscopin C (5) has no OH group, but instead two PhCOO groups at C_3- and C_7-positions (δ5.13 and 5.70), respectively. As expected, when treated with PhCOCl - pyridine (room temp., 10 h), 3 was readily converted into euphoscopin C (5) in 68% yield. Euphoscopin D (6), in which there is no OH group, has an AB quartet (δ3.02 and 4.50; C_8-H and C_8-H') and a broad doublet (δ6.77: C_5-H), indicating the presence of an additional CO group at C_7-position. Thus, oxidation of 3 with MnO_2 in benzene (70 - 80 °C, 24 h) afforded euphoscopin D, in which any enolization of the β-diketone system does not take place because of some unfaborable ring-strain. Application of molecular mechanics calculations to euphoscopins have also been carried out successfully.[10] In the case of 3, the most stable conformer based on molecular mechanics calculations is compatible with the result of an X-ray crystallographic analysis.[1,10]

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References and Notes

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2. Y. Shizuri, S. Kosemura, S. Yamamura, S. Ohba, M. Ito, and Y. Saito, Chem. Lett., 1983, 65.
3. Helioscopinolides and related diterpenes have also been isolated.
4. 8 as an oil: $C_9H_{14}O_2$[m/e 154.0976(M^+)]; IR (film) 1720,1635, 1600cm^{-1}; δ($CDCl_3$) 0.93(3H, t, J= 7Hz), 1.45(2H, tq, J= 7, 7Hz), 2.29(2H, dt, J= 7, 7Hz), 3.75(3H, s), 5.84(1H, dt, J= 10.5, 7Hz), 5.87(1H, d, J= 15Hz), 6.14(1H, dd, J= 10.5, 11Hz), 7.64(1H, dd, J= 11, 15Hz).
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6. 9 as an oil: $C_8H_{11}O_2$[m/e 139.0758(M^+ - OMe)]; IR (film) 1725, 1655cm^{-1}; δ($CDCl_3$) 0.95(3H, t, J= 6.5Hz), 1.2 - 1.7(4H, complex), 3.20(1H, dt, J= 4.5, 5Hz), 3.51(1H, dd, J= 4.5, 7 Hz), 3.78(3H, s), 6.13(1H, d, J= 15.5Hz), 6.84(1H, dd, J= 7, 15.5Hz).
7. 11 as an oil: $C_{22}H_{32}O_5$[m/e 376.2275(M^+)]; IR (film) 3450, 1720br., 1610br.cm^{-1}; δ($CDCl_3$) 1.03(3H, d, J= 7Hz), 1.12(3H, s), 1.21(3H, s), 1.52(3H, s), 1.81(3H, s), 2.01(3H, s), 2.06 - 2.73(4H, complex), 2.90(1H, br.s), 3.71(1H, br.s), 3.83(1H, dd, J= 2, 6Hz), 4.81(1H, dd, J= 3, 11Hz), 6.07(1H, br.d, J= 11Hz), 7.37(1H, br.d, J= 11Hz). 12: mp 196 - 201 °C; $C_{22}H_{32}O_5$[m/e 376.2285(M^+)]; IR (film) 3450, 1715br., 1610br.cm^{-1}; δ($CDCl_3$) 1.07(3H, s), 1.08(3H, d, J= 7Hz), 1.18(3H, s), 1.47(3H, d, J= 1.5Hz), 1.77(3H, s), 2.03(3H, s), 2.17 - 2.53(3H, complex), 2.71(1H, dd, J= 8, 10.5Hz), 3.11(1H, br.s), 3.96(1H, dd, J= 3.5, 10Hz), 4.80(1H, dd, J= 6, 8Hz), 5.82(1H, br.d, J= 10.5Hz), 7.26(1H, br.d, J= 11Hz).
8. 13 as an yellow oil: $C_{28}H_{36}O_4$[m/e 436.2602(M^+)]; IR (film) 1745, 1710, 1665, 1635, 1610cm^{-1}; δ($CDCl_3$) 0.92(3H, t, J= 7Hz), 1.10(3H, s), 1.17(3H, s), 1.23(3H, d, J= 7Hz), 1.58(3H, br.s), 1.86(3H, br.s), 2.01 - 2.65(8H, complex), 5.24(1H, br.d, J= 9Hz), 5.67 - 6.20(5H, complex), 7.61(1H, dd, J= 11, 15Hz).
9. J. Rhee, COORD program, QCPE, #226; N. L. Allinger and Y. H. Yuh, MMI/MMPI program (1973), QCPE, #318.
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12. 5 as an oil: $C_{38}H_{44}O_9$[m/e 644.2975(M^+)]; IR (film) 1715br., 1600, 1580cm^{-1}; δ($CDCl_3$) 0.93 (3H, d, J= 7Hz), 1.08(3H, d, J= 7Hz), 1.13(3H, s), 1.29(3H, s), 1.94(3H, d, J= 1.5Hz), 2.15 (3H, s), 2.19(3H, s), 2.38 - 3.50(5H, complex), 5.13(1H, dd, J= 2, 7Hz), 5.17(1H, dd, J= 7.5, 16Hz), 5.43(1H, d, J= 16Hz), 5.70(1H, dd, J= 4.5, 11Hz), 5.88(1H, br.d, J= 11Hz), 5.95(1H, d, J= 1.5Hz), 6.88 - 7.04(3H, m), 7.19 - 7.50(3H, m), 7.50 - 7.62(2H, m), 7.85 (2H, m). 6 as an oil: $C_{31}H_{38}O_8$[m/e 538.2545(M^+)]; IR (film) 1740, 1710, 1675, 1600, 1580 cm^{-1}; δ($CDCl_3$) 0.91(3H, d, J= 7Hz), 1.15(3H, s), 1.18(3H, d, J= 7Hz), 1.32(3H, s), 1.83 (3H, d, J= 1.5Hz), 2.15(3H, s), 2.22(3H, s), 2.35 - 2.62(2H, m), 3.02(1H, d, J= 15Hz), 3.11(1H, dd, J= 7.5, 15Hz), 3.27(1H, t, J= 7.5Hz), 4.50(1H, d, J= 15Hz), 5.27(1H, dd, J= 8, 15Hz), 5.32(1H, dd, J= 3, 7.5Hz), 5.55(1H, d, J= 15Hz), 5.88(1H, d, J= 1.5Hz), 6.77 (1H, br.d, J= 7.5Hz), 7.40 - 7.57(3H, m), 7.92(2H, m).

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