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STRUCTURAL AND CONFORMATIONAL STUDIES ON EUPHORNIN AND RELATED DITERPENES

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Summary: In addition to euphornin, three new toxic substances (euphornins A, B and C) have been isolated from the plant Euphorbia helioscopia L., and their absolute stereostructures also been elucidated on the basis of their spectral data and some chemical evidence together with an X-ray crystallographic analysis of the p-bromobenzoate derived from euphornin. Furthermore, their conformations have also been discussed.

In connection with highly oxygenated diterpenes which have antitumor activity or promote cancer development in tumor formation, we have isolated euphoscopins A and B from the plant Euphorbia helioscopia L.[1] Further investigation of toxic substances in the same plant resulted in the isolation of three new diterpenes (euphornins A, B and C) in addition to euphornin.[2,3]

As described in the previous paper,[1] 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 separated by column chromatography on silica gel (Mallinckrodt 100 mesh) using a gradient solvent of hexane - AcOEt (1 : 1~3) 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 give euphornin (1) as a main component (1.5 g) and euphornins A, B and C (2~4) (2, 13 mg; 3, 9 mg; 4, 49 mg). The physical data of these newly isolated diterpenes are shown below.

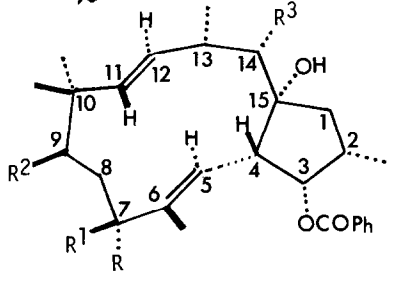
Euphornin A (2): mp 98 - 102 °C; m/z 525.2870 [$C_{31}H_{42}O_8$(M^+) - OH]; IR (film) 3470, 1710br., 1600, 1580 cm^{-1}; δ($CDCl_3$) 0.94(6H, s)(C_{10}-Me), 0.96(3H, d, J= 7Hz)(C_2-Me), 0.99(3H, d, J= 7 Hz)(C_{13}-Me), 1.68(3H, br.s)(C_6-Me), 2.05(3H, s)(AcO), 2.22(3H, s)(AcO), 2.97(1H, dd, J= 5, 10.5Hz)(C_4-H), 4.09(1H, br.d, J= 6Hz)(C_7-H), 4.37(1H, dd, J= 1.5, 4.5Hz)(C_9-H), 4.95(1H, d, J= 3Hz)(C_{14}-H), 5.05(1H, d, J= 16.5Hz)(C_{11}-H), 5.41(1H, dd, J= 4, 5Hz)(C_3-H), 5.63(1H, dd, J= 9, 16.5Hz)(C_{12}-H), 5.82(1H, br.d, J= 10.5Hz)(C_5-H), 7.4 - 7.6(3H, m) and 8.07(2H, m)(PhCOO).

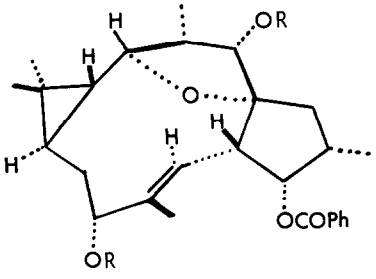
Euphornin B (3) as a colorless oil: $C_{31}H_{42}O_8$ [m/z 542.2879(M^+)]; IR (film) 3530, 1730br., 1600, 1580 cm^{-1}; δ($CDCl_3$) 0.85(3H, s)(C_{10}-Me), 0.95(6H, d, J= 6Hz)(C_2-, C_{13}-Me), 1.10(3H, s)(C_{10}-Me), 1.26(3H, s)(C_7-OAc), 1.72(3H, br.s)(C_6-Me), 2.21(3H, s)(C_{14}-OAc), 2.86(1H, dd, J= 4.5, 10Hz) (C_4-H), 3.34(1H, dd, J= 3, 3.5Hz)(C_9-H), 4.92(1H, br.d, J= 6Hz)(C_7-H), 4.92(1H, d, J= 3Hz) (C_{14}-H), 5.05(1H, d, J= 15Hz)(C_{11}-H), 5.36 - 5.74(3H, complex)(C_3-, C_5-, C_{12}-H), 7.4 - 7.6 (3H, m) and 8.06(2H, m)(PhCOO).

Euphornin C (4) as a colorless oil: $C_{31}H_{40}O_8$ [m/z 540.2682(M^+)]; IR (film) 3500, 1720br., 1600, 1580 cm^{-1}; δ($CDCl_3$) 0.91(3H, d, J= 6Hz)(C_2-Me), 0.93(3H, s)(C_{10}-Me), 0.97(3H, d, J= 6Hz) (C_{13}-Me), 1.15(3H, s)(C_{10}-Me), 1.81(3H, br.s)(C_6-Me), 2.01(3H, s)(AcO), 2.17(3H, s)(AcO), 2.85 (1H, dd, J= 4.5, 9Hz)(C_4-H), 3.23(1H, br.dd, J= 2, 13.5Hz)(C_8-H), 4.78(1H, dd, J= 2, 9Hz) (C_9-H), 4.93(1H, d, J= 2Hz)(C_{14}-H), 5.03(1H, d, J= 16.5Hz)(C_{11}-H), 5.49 - 5.74(2H, complex) (C_3-, C_{12}-H), 6.93(1H, br.d, J= 9Hz)(C_5-H), 7.3 - 7.5(3H, m) and 7.92(2H, m)(PhCOO).

In connection with euphoscopin A,[1] further structural study has been made on euphornin. In conclusion, the structure of euphornin, which has been proposed by Bohlmann and his co-workers,[2] must be revised, as follows.

When hydrolyzed with K_2CO_3 - MeOH (room temp., 10 h), euphornin (1) was converted into two deacetyl compounds (5 and 6)[4] in 70 and 19% yields, respectively. The former was further treated with 2,2-dimethoxypropane - TsOH in acetone to afford the corresponding acetonide (7)[5] in high yield, which was hydrolyzed again with K_2CO_3 - MeOH (room temp., 60 h) and then treated with p-bromobenzoyl chloride - pyridine to afford a p-bromobenzoate (8)[6] in 31% yield. This compound (8) was subjected to an X-ray crystallographic analysis, as follows.



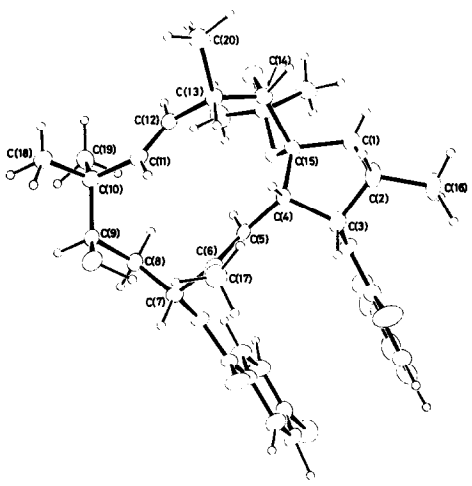


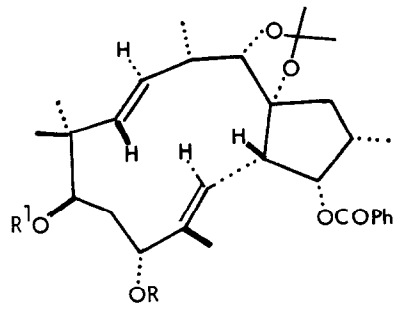
1 R = R^2= R^3= OAc, R^1= H
2 R = OH, R^1= H, R^2= R^3= OAc
3 R = R^3= OAc, R^1= H, R^2= OH
4 R R^1 = O, R^2= R^3= OAc
5 R = R^2= OAc, R^1= H, R^3= OH
6 R = R^2= R^3= OH, R^1= H

9 R = H
10 R = Ac

CRYSTAL DATA: $C_{37}H_{45}O_7Br$, MW 681.7, monoclinic, $P2_1$, a = 17.104(3), b = 13.837(2), c = 7.611 (1) Å, β = 99.14(2)°, Z = 2, V = 1778.2(5) $Å^3$, D_x= 1.27 g cm^{-3}, μ(Mo K_α) = 1.19 mm^{-1}.

X-ray intensity measurements were performed for 2θ(Mo K_α) $\leqq$ 55° on a Rigaku automated four-circle diffractometer with a crystal 0.3 x 0.3 x 0.5 mm in dimensions. 1793 Unique reflections were observed above the threshold [$|F_o| > 3\sigma(|F_o|)$]. Corrections were applied for Lorentz, polarization and absorption effects. The structure was solved by Patterson-Fourier methods and refined by block-diagonal least squares with anisotropic thermal parameters for all non-hydrogen atoms.[7] All H atoms are found on a difference map and included in the refinement with isotropic thermal parameters. The function minimized was $(\Sigma w||F_o| - |F_c||^2)$; weights were assigned as $w^{-1} = \sigma^2(|F_o|) + (0.015|F_o|)^2$. The final R was 0.039 and wR = 0.036 for 1793 unique reflections.[8] The enantiomorphic structure was also refined separately (R = 0.044, wR = 0.043) and it was rejected at the 0.005 significance level by the Hamilton test.[9] An ORTEP drawing is shown in Fig. 1 with the correct absolute configuration.[10,11]





7 R = R^1 = Ac

8 R = COC_6H_4Br-p, R^1 = H

Fig. 1 A computer generated ORTEP drawing of the molecule 8

Thus, the absolute stereostructure of euphornin must be represented by 1. The structures of the remaining three diterpenes were also determined on the basis of their spectral data coupled with some chemical evidence.

On acetylation with Ac_2O - pyridine, euphornins A and B (2 and 3) were readily converted into euphornin (1) in almost quantitative yields. Therefore, the stereostructures of these diterpenes are shown to be 2 and 3, respectively: the ^1H NMR spectrum of the former shows a broad doublet at δ 4.09 (C_7-H) and no Me singlet due to the AcO group at C_7-position usually observed in high magnetic field, while there are two remarkable signals at δ 1.27 and 4.92 (AcO-C_7-H) in the case of euphornin B (3). Finally, euphornin C (4) having a broad doublet at δ 6.93 (C_5-H) in its ^1H NMR spectrum was produced in 40% yield, on oxidation of 2 with MnO_2 in benzene (80 °C, 7 days).

As judged from the ^1H NMR spectra, conformations of euphornin (1) and euphoscopin A[1] are

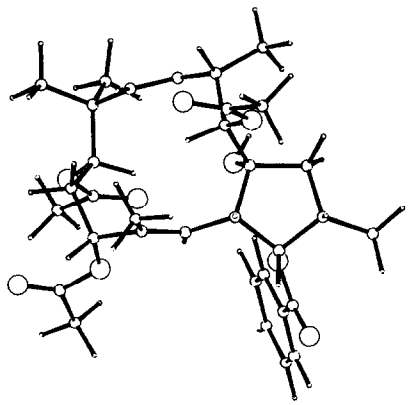


Fig. 2 The most stable conformation of euphornin (1) (steric energy: 51.0898 Kcal/mol)

Table 1

Proton	J (Calcd) (Hz)*	J (Found) (Hz)**
C_1-H, C_2-H	5.3 - 8.0	7
C_1-H', C_2-H	9.4 - 12.5	12
C_2-H, C_3-H	4.1 - 6.1	4.5
C_3-H, C_4-H	3.7 - 5.5	5
C_4-H, C_5-H	11.5 - 15.4	10
C_7-H, C_8-H	4.6 - 6.9	6.5
C_7-H, C_8-H'	0.8 - 1.2	1
C_8-H, C_9-H	9.7 - 12.2	3.5
C_8-H', C_9-H	0.0 - 0.0	3.5
C_{12}-H, C_{13}-H	6.9 - 9.2	8
C_{13}-H, C_{14}-H	1.5 - 2.2	3.5

* Based on the equations cited in the following literature: S. Sternhell, Quarterly Reviews, 23, 236 (1969).

** See ref. 2.

considerablly different to each other. The molecular mechanics calculations and ^{1}H NMR spectrum of the latter indicate that euphoscopin A adopts the most stable conformation similar to the stereostructure elucidated by an X-ray crystallographic analysis.[1] Although the coupling constants observed in 1 are roughly compatible with the corresponding ones calculated on the basis of the most stable conformer which is deduced by molecular mechanics calculations of 1 (see Table 1 and Fig. 2),[12] some remarkable differences are observed in J-values, strongly suggesting that the conformation of 1 is much flexible around C_8 and C_9 atoms.[13] In fact, the J-values of C_9-H varied with functional groups attached to the 12-membered ring. Interestingly, the coupling constants of C_9-H [δ4.78(1H, dd, J= 2, 9 Hz)] are in good agreement with the calculated ones in the case of euphornin C (4), in which the 12-membered ring is pretty rigid because of the presence of the CO group at C_7-position.

In the light of the plausible conformation depicted in Fig. 2, deacetyleuphornin (5) was treated with p-TsOH in acetone (room temp., 5.5 h) to afford a tricyclic compound (9)[14], in 76% yield, whose stereostructure was confirmed by its spectral data together with the following chemical evidence: acetylation of 9 with Ac_2O - pyridine yielded the corresponding diacetate (10)[15] in 74% yield. This is the first example, in which a jatrophone-type compound has been chemically transformed to a trans lathylane-type one, although such a trans tricyclic compound as 9 has not yet been found in nature.

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References and Notes

1. S. Yamamura, S. Kosemura, S. Ohba, M. Ito, and Y. Saito, Tetrahedron Lett., 22, 5315 (1981) and references cited therein.
2. R. Sahai, R. P. Rastogi, J. Jakupovic, and F. Bohlmann, Phytochemistry, 20, 1665 (1981).
3. The IR and ^{1}H NMR spectra of our sample were completely identical with those of euphornin. We are indebted to Prof. Bohlmann (Technical University, Berlin) for identification of our sample.
4. 5 as a colorless oil: $C_{31}H_{42}O_8$ [m/z 542.2871(M^+)]; IR (film) 3550, 1730br., 1600, 1580 cm^{-1}; δ($CDCl_3$) 3.32(1H, d, J= 3Hz)(C_{14}-H). 6 as a syrup: $C_{27}H_{38}O_6$ [m/z 458.2671(M^+)]; IR (film) 3400br., 1710, 1690, 1600, 1580 cm^{-1}; δ($CDCl_3$) 3.24(2H, complex)(C9-, C_{14}-H), 4.12(1H, br.d, J= 5Hz)(C_7-H).
5. 7 as a colorless oil: $C_{34}H_{46}O_8$ [m/z 582.3235(M^+)]; IR (film) 1740, 1725, 1600, 1590 cm^{-1}; δ($CDCl_3$) 1.66(6H, s)(acetonide), 4.08(1H, br.s)(C_{14}-H).
6. 8: mp 159 - 161 °C (from hexane); $C_{36}H_{42}O_7Br$ [m/z 665.2109(M^+- 15)]; IR (film) 3450, 1705, 1600, 1580 cm^{-1}.
7. The calculations were carried out with the Universal Crystallographic Computation Program System UNICS III: T. Sakurai and K. Kobayashi, Rikagaku Kenkyusho Hokoku, 55, 69 (1979).
8. Full details of all X-ray crystal structure determination will be published separately.
9. W. C. Hamilton, Acta Cryst., 18, 502 (1965).
10. C. K. Johnson, ORTEP. Report ORNL-3794, Oak Ridge National Laboratory, Tennessee, 1965.
11. Tables of atomic parameters, bond lengths and bond angles have been deposited with the Cambridge Crystallographic Data Centre.
12. J. Rhee, COORD program, QCPE,#226; N. L. Allinger and Y. H. Yuh, MMI/MMPI program (1973), QCPE,#318.
13. In the variable temperature ^{1}H NMR spectrum of 1 (in $CDCl_3$), which was taken on a JEOL FX-100 NMR spectrometer (100 MHz), the broad triplet due to C_9-H became sharp one gradually with an increase of temperature (30 - 85 °C).
14. 9 as a colorless oil: $C_{27}H_{36}O_5$ [m/z 440.2581(M^+)]; IR (film) 3450, 1705, 1600, 1580 cm^{-1}; δ($CDCl_3$) -0.16(1H, t, J= 5.5Hz)(C_{11}-H), 0.84(1H, dd, J= 5.5, 11Hz)(C_9-H), 3.54 (1H, t, J= 5.5Hz)(C_{12}-H), 4.00(1H, t, J= 5.5Hz)(C_{14}-H), 4.18(1H, t, J= 2Hz)(C_7-H).
15. 10 as a colorless oil: $C_{31}H_{40}O_7$ [m/z 524.2758(M^+)]; IR (film) 1740, 1720, 1600, 1580 cm^{-1}; δ($CDCl_3$) 1.33(3H, s)(C_7-OAc), 2.19(3H, s)(C_{14}-OAc), 5.11(1H, t, J= 3Hz)(C_7-H), 5.23(1H, d, J= 5Hz)(C_{14}-H).

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