Tetrahedron Letters,Vol.22,No.52,pp 5315-5318,1981 0040-4039/81/525315-04$02.00/0
Printed in Great Britain

THE ISOLATION AND STRUCTURES OF EUPHOSCOPINS A AND B

Shosuke Yamamura, Seiji Kosemura, Shigeru Ohba, Masatoki Ito, and Yoshihiko Saito
Department of Chemistry, Faculty of Science and Technology, Keio University,
Hiyoshi, Yokohama, Japan

Summary: Two new toxic substances, euphoscopins A and B, have been isolated from the plant Euphorbia helioscopia L., and their absolute stereostructures also been elucidated on the basis of their spectral data coupled with an X-ray crystallographic analysis of the p-bromobenzoate readily obtained on treatment of euphoscopin A with p-bromobenzoyl chloride - pyridine. These antitumor substances belong to a group of jatrophone-type diterpenes.

Many structural studies have been reported for antitumor activity on a number of polyoxygenated diterpenes including ingenol, phorbol, jatrophone and others.[1] Among them, however, only three substances having the jatrophone skeleton have been found in nature.[2] In addition, recently, Bohlmann and his co-workers isolated a new jatrophone-type diterpene, euphornin,[3] from the plant Euphorbia maddeni B., and proposed a tentative stereostructure for this substance, in which the configuration at the ring junction of the bicyclic carbon skeleton is cis. This structural study prompted us to report our new results concerning about the isolation and the absolute stereostructures of two new polyoxygenated diterpenes, euphoscopins A and B.

Fresh leaves and roots of the plant Euphorbia helioscopia L. (2.2 Kg) collected at Kanagawa, early in June, were immersed in MeOH at room temperature, and then the MeOH extract was concentrated and shaken with AcOEt. The AcOEt extract was directly chromatographed on silica gel (Mallinckrodt, 100 mesh). After elution of esters of higher fatty acids with $CHCl_3$, further elution with $CHCl_3$ - AcOEt (2 : 3) afforded a crude oil, which was further separated by repeating preparative TLC (kieselgel PF_{254}) using hexane - Et_2O (2 : 3), hexane - AcOEt (3 : 1) and/or $CHCl_3$ - AcOEt (10 : 1) to give two diterpenes, named euphoscopin A (1) and euphoscopin B (2) (1, 34 mg; 2, 77 mg). The physical data of these two substances are shown below.

Euphoscopin A as a colorless oil: $C_{31}H_{40}O_8$ [m/e 540.2706(M^+)]; ν_{max} (film) 3500, 1740, 1710, 1600 and 1580 cm^{-1}; ^1H NMR ($CDCl_3$): δ0.91(3H, d, J= 7Hz)(C_2-Me), 1.10(3H, d, J= 7Hz)(C_{13}-Me), 1.08(3H, s)(C_{10}-Me), 1.22(3H, s)(C_{10}-Me), 1.44(1H, dd, J= 9.5, 15Hz)(C_1-H), 1.82(3H, d, J= 1.5Hz)(C_6-Me), 2.15(3H, s)(AcO), 2.18(3H, s)(AcO), 2.2-2.6(2H, m)(C_2-H, C_{13}-H), 2.63(1H, dd, J= 5, 15.5Hz)(C_8-H), 2.99(1H, dd, J= 10.5, 15.5Hz)(C_8-H), 3.01(1H, dd, J= 7, 15Hz)(C_1-H), 3.29(1H, dd, J= 7, 9Hz)(C_4-H), 4.44(1H, dd, J= 5, 10.5Hz)(C_7-H), 5.12(1H, dd, J= 7.5, 15Hz)(C_{12}-H), 5.23(1H, dd, J= 3.5, 7Hz)(C_3-H), 5.36(1H, d, J= 15Hz)(C_{11}-H), 5.68(1H, qd, J= 1.5, 9Hz)(C_5-H), 5.93(1H, d, J= 1.5Hz)(C_{14}-H), 7.35-7.65(3H, m) and 7.99(2H, m)(C_6H_5COO).

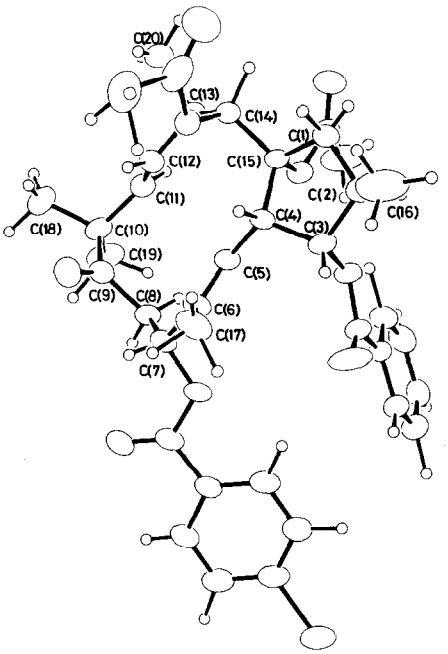
Euphoscopin B as a colorless oil: $C_{33}H_{42}O_9$ [m/e 582.2806(M^+)]; ν_{max} (film) 1735, 1720, 1600 and 1585 cm^{-1}; ^1H NMR ($CDCl_3$): δ0.92(3H, d, J= 7Hz), 1.10(3H, s), 1.10(3H, d, J= 7Hz), 1.25(3H, s), 1.26(3H, s), 1.43(1H, dd, J= 9.5, 15Hz), 1.85(3H, d, J= 1.5Hz), 2.14(3H, s), 2.20(3H, s), 2.2-2.6(2H, m), 2.63(1H, dd, J= 5, 15.5Hz), 2.8-3.1(2H, m), 3.24(1H, dd, J= 7, 9Hz), 5.08(1H, dd, J= 7.5, 15Hz), 5.11(1H, dd, J= 3.5, 7Hz), 5.31(1H, dd, J= 5, 10.5Hz), 5.33(1H, d, J= 15Hz), 5.61(1H, qd, J= 1.5, 9Hz), 5.88(1H, d, J= 1.5Hz), 7.25-7.6(3H, m) and 7.95(2H, m).

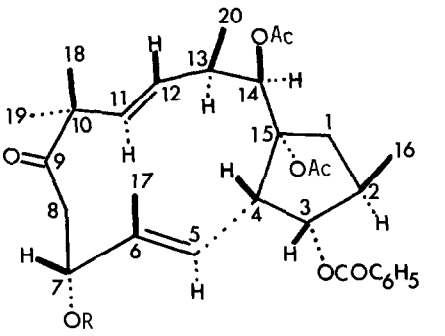
As seen in their spectral data, both diterpenes (1 and 2) are quite similar structurally to each other except for the following point. Euphoscopin A (1) has a molecular formula $C_{31}H_{40}O_8$ with one secondary OH group (ν_{max} 3500 cm^{-1}; δ4.44). On the other hand, euphoscopin B (2) with a molecular formula $C_{33}H_{42}O_9$ has an additional AcO group (δ1.26 and 5.31) instead of OH group, indicating that the latter must be the acetate derivative of euphoscopin A. As expected, on treatment with Ac_2O - pyridine (70 °C, 1 h), 1 was readily converted into 2. The ^1H NMR spectrum of euphoscopin A (1) with the aid of decoupling technique indicates the presence of following fragments: C_1~C_6, C_7-C_8 and C_{11}~C_{14} in addition to two AcO, one C_6H_5COO and two tertiary Me groups.

When treated with p-bromobenzoyl chloride - pyridine (room temp., overnight and then 60 °C, 1 h), euphoscopin A (1) was almost quantitatively converted into the corresponding p-bromobenzoate (3) as colorless prisms [mp 203 - 204 °C (from AcOEt - Acetone); $C_{38}H_{43}O_9Br$ (m/e 724.2108 and 722.2055(M^+)); ν_{max} (Nujol) 1740, 1720sh., 1710 and 1585 cm^{-1}], the ^1H NMR spectrum of which is quite similar to that of 1 except for the following point: the double doublet at δ4.44 in 1 was shifted to δ5.67 in 3. The stereostructure of 3 was finally determined by means of an X-ray

crystallographic analysis, as follows.

CRYSTAL DATA: $C_{38}H_{43}O_9Br$, MW 723.7, orthorhombic, $P2_12_12_1$, a = 16.271(2), b = 23.577(3), c = 9.786(1) Å, Z = 4, V = 3754.1(6) Å^3, D_x = 1.28 g·cm^{-3}, μ(Cu Kα) = 2.07 mm^{-1}.





1 R = H

2 R = Ac

3 R = p-BrC$_6$H$_4$CO

Fig. 1 A computer generated ORTEP drawing of the molecule 3

The intensity measurements were performed for $2\theta \leq 135°$ on a Rigaku automated four-circle diffractometer with graphite-monochromated Cu Kα radiation, a θ-2θ scan technique, and a crystal 0.52 x 0.26 x 0.26 mm. 3591 Intensities with $|F_o| \geq 3\sigma(|F_o|)$ were considered as observed. LP corrections and a numerical absorption correction were applied. The structure was solved by Patterson-Fourier methods and refined by block-diagonal least-squares with anisotropic thermal parameters for all non-hydrogen atoms.[4] The H atoms are found on a difference map and included in the refinement. The function minimized was $(\Sigma w||F_o| - |F_c||^2)$; weights were assigned as $w^{-1} = \sigma_F^2(\text{count.}) + (0.015|F_o|)^2$. The final R was 0.043 and R_w = 0.056.[5] The enantiomorphic structure was also refined separately (R = 0.053, R_w = 0.069) and it was rejected at the 0.005 significance level by the Hamilton test.[6] An ORTEP drawing is shown in Fig. 1 with the correct absolute configuration.[7,8] Accordingly, the absolute stereostructures of euphoscopins A and B should be represented by 1 and 2, respectively.

As judged from the ^{1}H NMR spectrum of 1, euphoscopin A in $CDCl_3$ seems to adopt the similar

conformation as shown in Fig. 1. Furthermore, in the case of 2, the sharp singlet due to the AcO group at C_7-position is observed in higher magnetic field (δ1.26). This can be explained well by anisotropic effect of the benzoate moiety at C_3-position, if 2 adopts the same conformation as shown in Fig. 1. Finally, structural similarity between euphoscopin A (1) and euphornin[3] suggests another possibility in which the latter has the same configuration as those of 1 at C_4- and C_{15}-positions. Further study on the configuration is in progress.

The authors wish to thank Mr. S. Kidokoro (Ohmori Research Institute, Tobishi Pharmaceutical Co. Ltd.) and Dr. Y. Shizuri (Nagoya University) for measurements of high resolution mass spectra as well as of NMR spectra. They are also indebted to Institute for Molecular Science for the use of the diffractometer and the HITAC M-180H computer.

References and Notes

1. Y. Hirata, Pure Appl. Chem., 41, 175 (1975); E. Hecker, Pure Appl. Chem., 49, 1423 (1977); W. Adolf and E. Hecker, Experientia, 34, 679 (1978); F. J. Evans and C. J. Soper, Lloydia, 41, 193 (1978) and many references cited therein.
2. S. M. Kupchan, C. W. Siegel, M. J. Matz, J. A. S. Renauld, R. C. Haltiwanger, and R. F. Bryan, J. Am. Chem. Soc., 92, 4476 (1970); D. Uemura and Y. Hirata, Tetrahedron Lett., 1975, 1697; D. Uemura, C. Katayama, E. Uno, K. Sasaki, and Y. Hirata, Tetrahedron Lett., 1975, 1703.
3. R. Sahai, R. P. Rastogi, J. Jakupovic, and F. Bohlmann, Phytochemistry, 20, 1665 (1981).
4. 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).
5. Full details of all X-ray crystal structure determination will be published separately.
6. W. C. Hamilton, Acta Cryst., 18, 502 (1965).
7. C. K. Johnson, ORTEP. Report ORNL-3794, Oak Ridge National Laboratory, Tennessee, 1965.
8. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre.

(Received in Japan 11 September 1981)