[Chem. Pharm. Bull. 22(4) 971—972 (1974)]

UDC 547.597.02 : 581.192

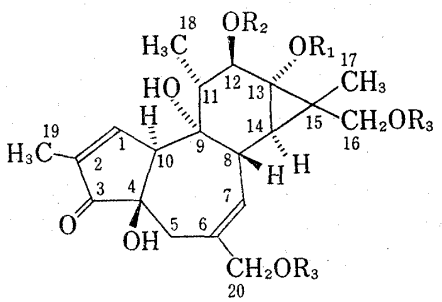
The Toxic Constituent of the Fruits of *Aleurites fordii*

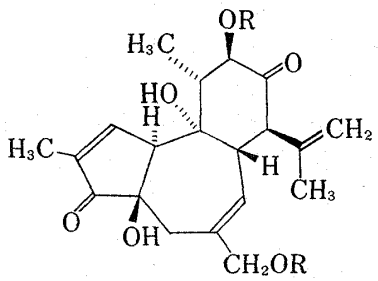
The fruits of *Aleurites* species (Euphorbiaceae) which provide tung oil are known toxic and have been the subject of a number of investigations,[1] but no toxic principle of detailed structure has been obtained. We have now isolated a crystalline toxic principle from the fruits of *Aleurites fordii* Hemsl. (Japanese name: Shinaaburagiri)

Seeds and residual part of the fruits were extracted with methanol separatedly. The constituent toxic to killie-fish was isolated from the latter after chromatography on silicic acid column as crystals (I), mp 177—178°, $C_{38}H_{60}O_9$ (M^+ 660), $[\alpha]_D^{12}$ +43° (c=0.30, MeOH). Another crystalline constituent (II), mp 278—282° (decomp.), $C_{22}H_{30}O_8$ (M^+ 422), $[\alpha]_D^{24}$ +65° (c=0.11, EtOH), was isolated from the former. Compound (I) might be palmitate of II as methyl palmitate was produced and identified by GC-MS upon methanolysis of (I), and tetra-acetate, $C_{28}H_{36}O_{11}$, of the alcoholic residue was found identical with triacetate of II.

The hydroxyl and α,β-unsaturated carbonyl absorptions are shown by the infrared (IR) and ultraviolet (UV) spectra of II, ν_{max} (KBr): 3505, 3500, 3400, 3250, 1705, 1695 (shoulder) and 1630 cm^{-1}, λ_{max} (MeOH): 232 nm (log ε 3.70). An acetyl group is shown by the nuclear magnetic resonance (NMR) (90 MHz, C_5D_5N, δ 2.02) and mass (M-60) spectra, and other NMR signals, δ 1.53 (3H, d, J=6 Hz), 1.57 (3H, s)[2], 1.70 (3H, dd, J=2, 1 Hz), 2.83 (1H, dq, J=10, 6 Hz), 3.04 (2H, s), 3.69 (1H, m), 3.97 (t, J=5, 6 Hz), 4.31 (2H, s), 4.20, 4.42 (2H, ABq, J=12 Hz), 4.49 (1H, d, J=10 Hz), 6.20 (1H, d, J=6 Hz), 7.81 (1H, diffused s), indicate analogy of II with phorbol.[3,4] The NMR spectrum in $(CD_3)_2SO$ shows presence of two tertiary, a secondary, and two primary hydroxyl groups in II.

The difference between II and phorbol is shown in the NMR spectrum as follows. One of the geminal methyl groups on the cyclopropane ring in phorbol is replaced in II by an AB quartet (J=12 Hz) at δ 4.20 and 4.42, which is assignable to a hydroxymethyl group. Upon





I: R_1=$COCH_3$, R_2=$CO(CH_2)_{14}CH_3$, R_3=H
II: R_1=$COCH_3$, R_2=R_3=H
III: R_1=R_2=R_3=$COCH_3$
VI: R_1=R_3=$COCH_3$, R_2=$CO(CH_2)_{14}CH_3$

IV: R=H
V: R=$COCH_3$

Chart 1

1) R.L. Holmes and E.T. Rayner, *J. Amer. Oil. Chem. Soc.*, **35**, 586 (1958); R.L. Holmes and R.T. Oćonnar, *ibid.*, **38**, 520 (1961).
2) A proton overlaps with these two methyl signals. The former is shown as a doublet (J=5 Hz) at δ 1.13 in $(CD_3)_2SO$.
3) E. Hecker, H. Bartsch, M. Gschwendt, E. Harle, G. Kreibich, H. Kubinyi, H.U. Schairer, Ch. v. Szczepanski and H.W. Thielmann, *Tetrahedron Letters*, **1967**, 3165; L. Crombie, M.L. Games and D.J. Pointer, *J. Chem. Soc.*, (*C*), **1968**, 1347.
4) R.C. Pettersen, G. Furguson, L. Crombie, M.L. Games and D.J. Pointer, *Chem. Commun.*, **1967**, 716; E. Hecker, H. Bartsch, G. Kreibich and Ch. v. Szczepanski, *Angew. Chem.*, **79**, 824 (1967); *idem*, *Ann.*, **725**, 130 (1969).

acetylation with Ac_2O–C_5H_5N, II yielded triacetate (III), $C_{28}H_{36}O_{11}$, whose NMR spectrum shows three newly formed acetyl groups, among which two are on the primary (δ 4.31→4.66 or 4.77; 4.42 and 4.20 ABq→4.77 s or 4.66 s), and one is on the secondary (δ 4.49→6.05) hydroxyl group. These observations lead to the assumption that II is an acetate of 16-hydroxyphorbol. The tertiary hydroxyl group at C-13 in phorbol is acetylable with Ac_2O–C_5H_5N.[3,5] Therefore, the acetyl group in II would be at C-13, since if the acetyl group is on one of the other tertiary hydroxyl groups, an additional acetyl group would be formed in III. This structure of II has further been supported by NMDR.

On the treatment of II with 0.02N H_2SO_4 in aqueous methanol, IV, $C_{20}H_{26}O_6$ (M^+ 362), mp 244—248° (decomp.), CD ($\Delta\varepsilon$) 284 nm (+2.21) and 345 nm (−1.29) (c=0.125, dioxane), λ_{max} (MeOH) 245 nm (log ε 3.65), was produced. The NMR spectrum shows that the methyl group on the cyclopropane ring, and C_{16}-methylene (ABq) group in II are replaced by an isopropenyl group ($(CD_3)_2SO$, olefinic methyl signal at δ 1.54 and vinyl signal at δ 4.75 and 4.92) in IV. Elimination of the acetoxyl group upon this transformation is also exhibited. Acetylation of IV with Ac_2O–C_5H_5N yielded diacetate (V), $C_{24}H_{30}O_8$ (M^+ 446), mp 191—192°, CD ($\Delta\varepsilon$) 292 nm (+1.67) and 348 nm (−0.73) (c=0.105, dioxane). The NMR spectrum of this diacetate was found identical with that of bisdehydrophorbol-12,20-diacetate,[6] and the identification with the authentic sample was done by mixed melting point, and IR, optical rotatory dispersion (ORD) and circular dichroism (CD) spectra to establish the structure and the absolute configurations (except C-15) of II as 13-O-acetyl-16-hydroxyphorbol.

The NMR spectrum of I is practically identical with that of II except the presence of a palmityl signal (3H, δ 0.88, m; 26H, δ 1.25, s; 2H, δ 2.40, t) and the downfield shift of C-12 proton by 1.56 ppm to δ 6.05 (d, J=10 Hz) in I. A diacetate (VI), $C_{42}H_{64}O_{11}$ (M^+ 744) was produced from I by Ac_2O–C_5H_5N to show that the two acyl groups in I are located at C-12 and C-13. The mass spectrum of I exhibits loss of $CH_3(CH_2)_{14}COO\cdot$ and CH_3COOH to indicate their respective locations at C-12 and C-13 rather than the reverse, based on the fragmentations of phorbol diesters which are known to give M—RCOO· ion by the elimination at C-12, and M—RCOOH ion by the elimination at C-13.[7] The intermediate product of the selective methanolysis[7] of I, which moved slower than I on thin-layer chromatography (TLC), was purified by preparative TLC and acetylated to give a product which was identified with VI by TLC and mass spectrography. This result shows that the palmityl group is retained in the intermediate product of the methanolysis, and that its location is at C-12 since it is known that the acyl group at C-13 on the phorbol skeletons is methanolyzed with MeONa–MeOH in advance to that at C-12. The structure of the toxic principle, therefore, is shown to be 12-O-palmityl-13-O-acetyl-16-hydroxyphorbol (I). The absolute configurations would also be represented by I because of the identity of ORD curves of III, ($[\phi]^{12}_{305}$ $+4.02° \times 10^3$ (c=0.15, MeOH), derived from I and II.

Acknowledgement We thank Professor Dr. E. Hecker for his generous supply of the sample of bisdehydrophorbol-12,20-diacetate.

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Received December 14, 1973

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