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Isolation and Structure of Phlegmanol F

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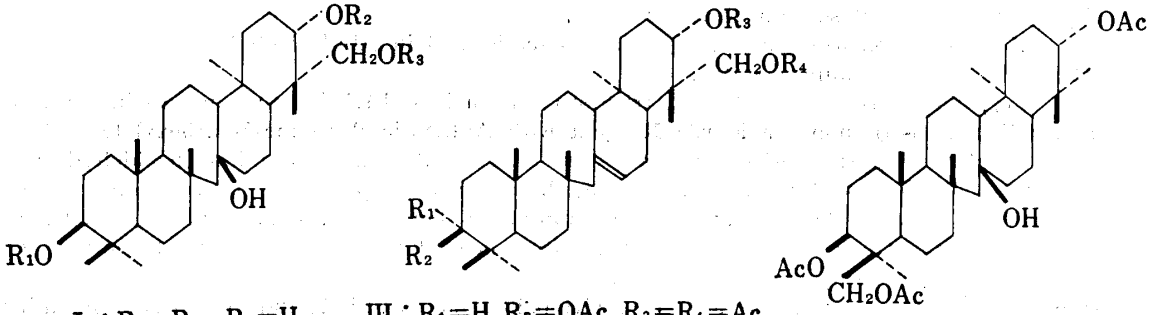
In the previous papers,[2,3] we reported isolations and structures of six new triterpenoids, phlegmanol A,B,C,D,E, and phlegmaric acid, all of which belong to the serratenediol family, from *Lycopodium phlegmaria* L. growing in Ceylon. Phlegmanol F is another new triterpene isolated from the same plant. In this note, we wish to describe the isolation and structure elucidation of phlegmanol F.



I : $R_1=R_2=R_3=H$
II : $R_1=R_2=R_3=Ac$
III : $R_1=H, R_2=OAc, R_3=R_4=Ac$
IV : $R_1=R_2=O, R_3,R_4=:C(CH_3)_2$
V : $R_1=R_2=O, R_3=R_4=Ac$
VI

Chart 1

Phlegmanol F (I) was isolated as its triacetate (II), $C_{36}H_{58}O_7$, mp 254—256°, $[\alpha]_D^{16}$ +16° (c=0.5, $CHCl_3$). The triacetate revealed nuclear magnetic resonance (NMR) signals due to ptotons geminal to acetoxyl groups at 4.26 (2H, ABq., δ_{AB}=19 Hz, J=12 Hz) and 4.57 (2H, broad m., $W_{1/2}$=18 Hz) as well as six tertiary methyl and three acetyl methyl signals but no signal corresponding to the olefinic proton and the proton geminal to a hydroxyl group was observed. The infrared (IR) spectrum of the triacetate showed the strong hydroxyl band at 3500 cm^{-1}. These spectral data suggested that phlegmanol F is a tetrahydroxyl triterpene with one primary, two secondary and one tertiary hydroxyl groups. The broad signals due to two protons, each of which is geminal to an acetoxyl group, suggested an equatorial orientation of two secondary hydroxyl groups and an axial orientation of the primary hydroxyl group was suggested from the chemical shift of a band due to -CH_2OAc which is in agreement with that for axial -CH_2OAc (4.30—4.08) rather than equatorial one (3.84—3.77).[4] Thus, phlegmanol F triacetate appears to be identical with tohogeninol triacetate (VI).[5,6] These two compounds, however, were proved to differ from one another by direct comparison.

The masss spectral fragmentation of triterpenes related to serratenediol have been reported by Kutney, *et al.*[7] As shown in Chart 2, the mass spectra of anhydrophleganol F keto-acetonide

1) Location: *a*) *Yoshida-Shimoadachi-cho, Sakyo-ku, Kyoto*; *b*) *Peradeniya, Ceylon.*
2) Y. Inubushi, T. Harayama, T. Hibino and R. Somanathan, *Chem. Commun.*, **1970**, 1118.
3) Y. Inubushi, T. Hibino, T. Harayama, T. Hasegawa and R. Somanathan, *J. Chem. Soc.* (*C*), **1071**, 3109.
4) A. Gaudemer, M.J. Polonsky and E. Wenkert, *Bull. Soc. Chim. France*, **1964**, 407.
5) Y. Inubushi, Y. Tsuda and T. Sano, *Chem. Pharm. Bull.* (Tokyo), **13**, 750 (1965).
6) T. Sano, T. Tsuda and Y. Inubushi, *Tetrahedron*, **26**, 2981 (1970).
7) J.P. Kutney and G. Eigendorf, *Tetrahedron*, **25**, 3753 (1969).

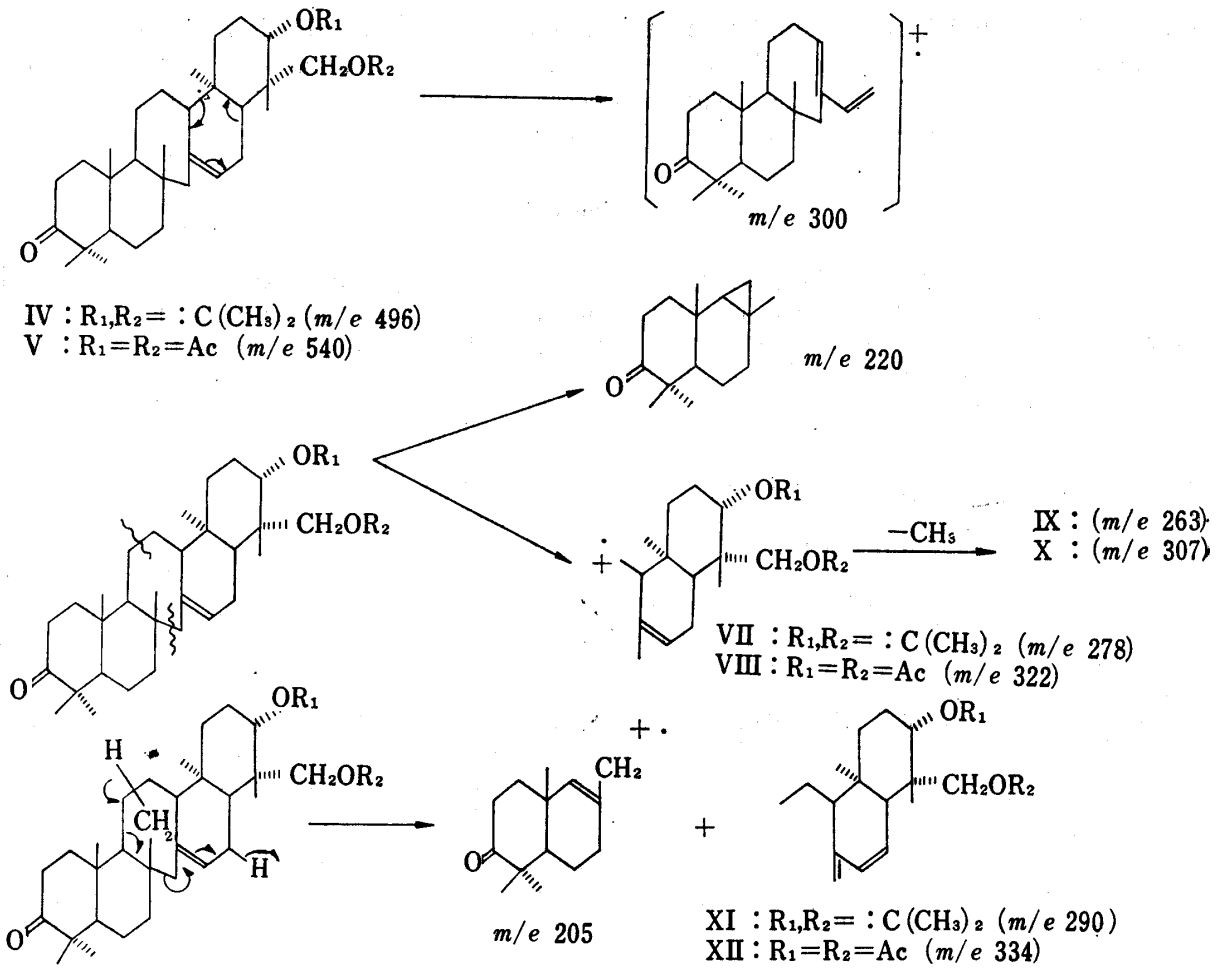


Chart 2

(IV) and keto-diacetate (V) (*vide infra*) revealed characteristic fragmentations for serratenediol type triterpenes, suggesting that phlegmanol F possesses the serratane skeleton.

On the other hand, all triterpenes of serratenediol series reported hitherto have the oxygen functions at the C_3 and C_{21} position. It seems, therefore, reasonable to assume that two secondary hydroxyl groups are situated at C_3 and C_{21}, respectively. When heated with 2,2-dimethoxypropane in DMF under the presence of *p*-TsOH catalyst, followed by oxidation with CrO_3-pyridine, phlegmanol F formed the anhydrophlegmanol F keto–acetonide (IV), mp 218—220° which showed no hydroxyl absorption band in the IR spectrum and revealed the signal corresponding to one olefincic proton in the NMR spectrum. This finding indicates that dehydration of phlegmanol F took place readily by acid (*p*-TsOH) treatment to give the keto–acetonide possessing the serratene skeleton. The compound (IV) showed NMR signals at 3.67 (2H, ABq., J=12 Hz, δ_{AB}=49 Hz, -CH_2-O-C<), 3.47 (1H, m., >CH-O-C<), 1.38 and 1.43 (each 3H, s., >$C(CH_3)_2$). This NMR spectral observation indicates that the acetonide linkage in the compound (IV) was formed between the axial primary and equatorial secondary hydroxyl group.[8] Therefore, the primary hydroxyl group forming the acetonide with the secondary hydroxyl group should be situated at the C_4 or C_{22} position. Since the optical rotatory dispersion (ORD) curve of the keto–acetonide (IV) showed a positive Cotton effect,[8] the ketonic function is located in the A ring. Thus, the axial primary hydroxyl group is situated at the C_{22} position and this conclusion was also supported by the mass spectral fragmentations of the compound IV and V as shown in Chart 2. From the foregoing results, the tertiary hydroxyl group of phlegmanol F should be situated at C_{14} and its configuration will be β

8) Y. Tsuda, T. Sano, A. Morimoto and Y. Inubushi. *Tetrahedron Letters*, **47**, 5933 (1966).

because phlegmanol F reacts similary to tohogenol[5] both in dehydration with ease by acid treatment to afford the serratene derivative. Consequently, phlegmanol F can be represented by the formula I. In view of the isolation process, it is possible that phlegmanol F exists in nature as the partially or fully acetylated compound.

Experimental

Mps were determined with a microscopic hot-stage and are uncorrected. Unless otherwise stated, the IR spectra were measured for solutions in $CHCl_3$ with a Hitachi EPI spectrometer and the NMR spectra were recorded at a Varian A-60 instrument, using $CDCl_3$ as the solvent; the line positions or centers of multiplets are given in the δ ppm scale with reference to TMS as the internal standard. The mass spectral determinations were performed with a Hitachi RMU-60 mass spectrometer with a direct heated inlet system. The column chromatography was performed on a) acid washed alumina (Brockmann alumina, Activity II—III, was treated with 5% aqueous acetic acid solution and dried at 130° for 2 hr) and b) Brockmann alumina Activity II—III, respectively, and the preparative thin-layer chromatography (TLC) was carried out on Kiesel gel GF_{254} nach Stahl; developing solvent; $CHCl_3$: acetone (15:1).

Extraction——The air-dried aerial parts of *Lycopodium phlegmaria* L. (5 kg) collected in Ceylon were extracted with hot methanol several times and the solvent was evaporated *in vacuo*. The residue was digested with 5% aqueous acetic acid solution to leave the neutral and acidic portions (360 g). These were successively extracted with *n*-hexane, benzene and $CHCl_3$ in a Soxhlet extractor. The *n*-hexane extract (69 g), the benzene extract (77 g), the $CHCl_3$ extract (142 g) and the residue (72 g) were obtained and the residue was treated as follows.

Isolation of Phlegmanol F Triacetate (II)——The residue (100 g) remained in the Soxhlet extractor was dissolved in 200 ml of dioxane and to this solution was added 200 ml of 10% KOH solution in methanol. The mixture was heated under reflux for 4 hr and after cooling, the mixture was concentrated and poured into ice water. The precipitates were collected by filtration, dried and acetylated with acetic anhydride–pyridine at room temperature. The reaction mixture was then worked up in the usual manner and the acetates (75 g) in $CHCl_3$ were chromatographed on alumina[b] (1 kg) and elution with $CHCl_3$ (Fraction No. 3—8, each fraction 500 ml) left the eluate (6.68 g). The eluate in benzene was rechromatographed on alumina[a] (130 g) and elution with the benzene–$CHCl_3$ (7:3) mixture (Fraction No. 7—10, each fraction 100 ml) gave the crystalline mass (200 mg) which was subjected to the repeated preparative TLC. Recrystallizations from $CHCl_3$–MeOH afforded phlegmanol F triacetate (II) as needles (167 mg), mp 254—256°, $[\alpha]_D^{16}$ +16° (c =0.5, $CHCl_3$). *Anal.* Calcd. for $C_{36}H_{58}O_7$: C, 71.44; H, 9.48. Found: C, 71.74; H, 9.70. IR ν_{max}^{KBr} cm^{-1}: 3500, 1725 and 1245. NMR: 0.83 (9H, s.), 0.95, 0.97, 1.00 (each 3H, s.), 2.03 (9H, s.), 4.26 (2H, ABq., J=12 Hz, δ_{AB}=19 Hz), 4.57 (2H, m., $W_{1/2}$=18 Hz).

Anhydrophlegmanol F Keto-acetonide (IV)——A solution of 124 mg of II in 12 ml of 5% methanolic KOH was refluxed on a water bath for 3 hr. The mixture was then concentrated and diluted with water. The precipitated phlegmanol F (88 mg), mp 315—320° was collected by filtration. To a solution of 88 mg of phlegmanol F in 5 ml of DMF were added 5 ml of 2,2-dimethoxypropane and 30 mg of *p*-TsOH, and the mixture was refluxed for 2 hr. After cooling, the reaction mixture was neutralized by addition of solid $NaHCO_3$ and then evaporated *in vacuo* to leave 100 mg of the residue. The residue in benzene was chromatographed on alumina[b] and the eluate with benzene was, without purification, oxidized with CrO_3-pyridine complex prepared from 100 mg of CrO_3 and 4 ml of pyridine. The reaction mixture was stirred at room temperature overnight, poured onto ice-water and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water and dried over anhyd. $MgSO_4$. The solvent was evaporated off to leave the curde IV which in benzene was chromatographed on alumina.[b] The eluate with benzene was recrystallized from MeOH to yield IV as needles (28 mg), mp 218—220°. IR ν_{max} cm^{-1}: 1700. NMR: 0.82, 1.03, 1.08, 1.22, 1.38, 1.43 (each 3H, s.), 0.88 (6H, s.), 3.47 (1H, m.), 3.67 (2H, AB_q., J=12 Hz, δ_{AB}=49 Hz), 5.33 (1H, m.). Mass Spectrum: *m/e* 496 (M^+, 3), 481 (M^+ -CH_3, 58), 438 (base peak), 263 (10), 269 (18), 205 (98). $[\Phi]_{308}^{dioxane}$ +3860° (peak) and $[\Phi]_{265}^{dioxane}$ −4364° (trough).

Anhydrophlegmanol F Keto-diacetate (V)——To 19 mg of IV was added 10 ml of 3% ethanolic HCl solution and the mixture was heated on a water bath for 30 min, and then evaporated to dryness *in vacuo*. The residue was acetylated with acetic anhydride-pyridine in the usual manner and the crude diacetate was recrystallized from MeOH to give the keto-diacetate (V) as needles (7 mg), mp 216—7°. *Anal.* Calcd. for $C_{34}H_{52}O_5$: C, 75.51; H, 9.69. Found: C, 75.44; H, 9.40. NMR: 0.72, 0.87, 0.90, 1.00, 1.04, 1.08 (each 3H, s.), 2.05 (6H, s.), 4.33 (2H, ABq., J=12 Hz, δ_{AB}=21 Hz), 4.40—4.83 (1H, m.) and 5.37 (1H, m.). Mass Spectrum *m/e*: 540 (M^+, 10), 480 (M^+-$C_2H_4O_2$, 97), 420 (M^+-2×$C_2H_4O_2$, 98), 405 (M^+-2×$C_2H_4O_2$-CH_3, 99), 340 (80), 334 (2), 322 (4), 307 (2), 300 (2), 261 (94), 220 (19), 218 (base peak), 205 (79).