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STRUCTURE OF PHARBITIC ACID, A GIBBERELLIN-RELATED DITERPENOID

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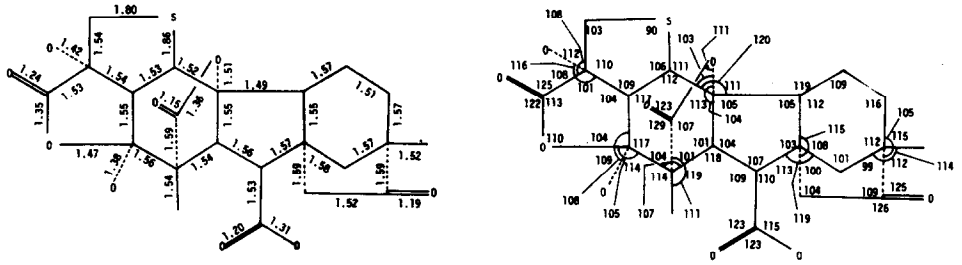
We have recently isolated a new diterpenoid[1], which we name pharbitic acid, from immature seed of Japanese morning-glory (Pharbitis nil) and have elucidated its structure closely related to gibberellin A_3 based on X-ray crystallographic analysis of its derivative obtained by acid treatment.

Pharbitic acid $C_{22}H_{24}O_9S$, d.p. 234-238°, showed i.r. absorptions at $\nu_{nujol}^{cm^{-1}}$ 3505, 3440, 3330 (hydroxyls), 1806, 1766, 1709 (carbonyls), 1659 (exocyclic methylene). Treatment of its acetone solution with ethereal diazomethane gave amorphous dimethyl ester, which showed n.m.r. (60 MHz) absorptions δ^{CDCl_3} 1.16 (3H s, tert. Me), 3.71, 3.78 (3H s, COOMe), 2.80, 3.70 (each 1H d J=10Hz, $\blacksquare\!>\!CH\text{-}HC\!<\!\blacksquare$), 3.05, 3.49 (each 1H d J=12Hz, $\blacksquare\text{-}CH_2\text{-}S$), 3.50, 4.49 (each 1H d J=7.5Hz, $\blacksquare\!>\!CH\text{-}HC\!<\!\overset{S}{\blacksquare}$), 4.97, 5.25 (each 1H br. s, $\blacksquare\!>\!C\!=\!CH_2$). Since some of these spectral data are characteristic of gibberellins, pharbitic acid was considered to be closely related to gibberellin.

Pharbitic acid was boiled in 1N HCl to yield a ketoacid $C_{22}H_{24}O_9S\cdot H_2O$, m.p. 195-200°, which shows the following spectral properties: $\nu_{nujol}^{cm^{-1}}$ 3510, 3240 (hydroxyls), 1788, 1770, 1728 (carbonyls); $\delta^{d_6\text{acetone}}$ 1.01, 1.36 (3H s, tert.Me), 2.20, 2.83 (1H d J=18Hz, 1H dd J=3, 18Hz, $\blacksquare\text{-}CH_2CO\text{-}$), 2.83, 3.57 (each 1H d J=6.3 Hz, $\blacksquare\!>\!CH\text{-}HC\!<\!\blacksquare$), 3.32 (2H br. s, $\blacksquare\text{-}CH_2\text{-}S$), 3.03, 4.40 (each 1H d J=8Hz, $\blacksquare\!>\!CH\text{-}HC\!<\!\overset{S}{\blacksquare}$). These data revealed the presence of new methyl and ketone groups formed by acid treatment, indicating that the ketoacid was derived via Wagner-Meerwein rearrangement, as in 13-hydroxygibberellins[2]. Recrystallization from acetone-n-hexane afforded prismatic crystals (monohydrate), suitable for X-ray

analysis, which belong to the orthorhombic system with space group $P2_12_12_1$ and the lattice parameters are: a=9.764, b=23.417, c=9.592 Å, V=2216.0 Å^3. The density was measured in KI-NaCl satd. solution to be Dm=1.443 g·cm^{-3}, indicating four monohydrate molecules are contained in a unit-cell (calcd. density, Dx= 1.444 g·cm^{-3}).

Intensities were measured by a Rigaku four-circle X-ray diffractometer with MoKα radiation obtained by a monochromator. A total of 2054 independent structure factors out of 3638 theoretically possible ones were obtained in the range $2\theta \leqq 60°$. The sulfur position was easily determined from Patterson synthesis. Fourier synthesis phased by the sulfur atom contributions followed by repeated cycles of Fourier and least squares refinements revealed all the 22 carbon and 10 oxygen atoms. The final R-value for the 2054 structure factors was 0.122. The bond lengths and angles are shown in Figs. 1 and 2. The average standard deviations for C-C lengths and C-C-C angles are 0.01 Å and 1°, respectively.



Figs. 1 and 2. The bond lengths (Å) and angles (°) of the ketoacid molecule.

The C.D. data of pharbitic acid (I), dimethyl pharbitate (II), ketoacid (III) and its dimethyl ester (IV) are shown in Fig. 3. The comparison of C.D. data between acids and respective dimethyl esters shows that even in solution the ketoacid exists almost in a lactol form. The C.D. value of the ketoacid θ^{EtOH}_{300nm}=-6920, which therefore must be mainly responsible for the C16 ketone, closely resembles the value θ^{EtOH}_{300nm}=-7540 for the C/D ring rearranged product of gibberellin A_{20}. Thus the absolute configuration of the ketoacid molecule is as depicted in III and Fig. 4.

Pharbitic acid and ketoacid have enolizable site at C2. The 70 hours ex-

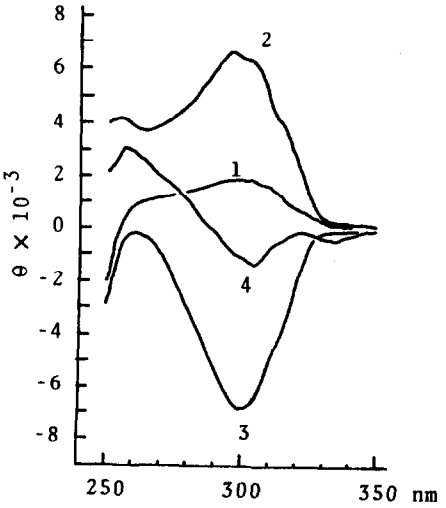


Fig. 3. C.D. spectra. 1, pharbitic acid (I). 2, dimethyl pharbitate (II). 3, ketoacid (III). 4, its dimethyl ester (IV). 1, 2 and 4 in MeOH, 3 in EtOH.

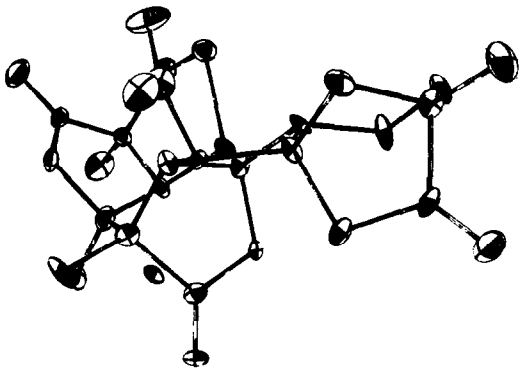
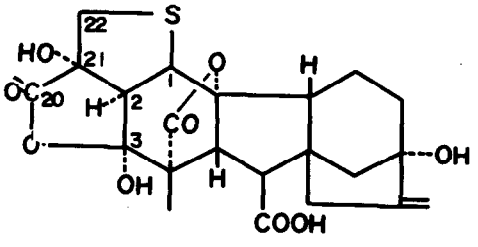
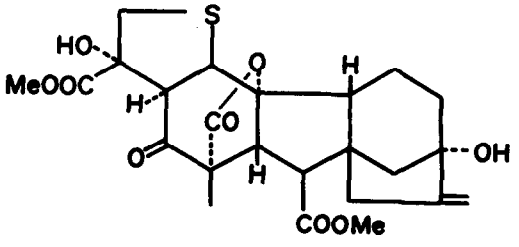


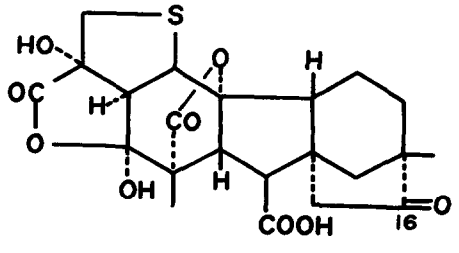
Fig. 4.



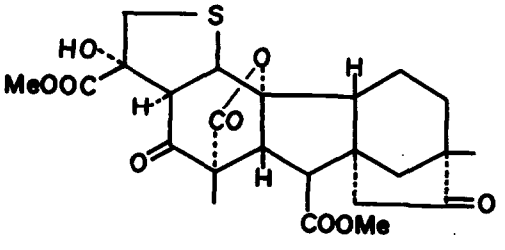
I



II



III



IV

posure of pharbitic acid in deuteromethanol resulted in 40% incorporation of deuterium at the C2 without epimerization at this center. This indicates that no epimerization of the C2 center also occurred in the conversion of pharbitic acid to ketoacid. Dimethyl pharbitate showed a positive C.D. maximum due to the C3 ketone Θ_{300nm}^{MeOH}=6140, which also indicates that the C2-C21 bond is β-oriented since the sign should be controlled by the sulfur, which can exist in positive octant only in the case of β-oriented C2-C21 bond. Thus the absolute structure of pharbitic acid is shown to be I.

Apparently pharbitic acid is biogenetically formed by oxidation of gibberellin A_3 accompanied by addition of mercaptopyruvic acid or cysteine to the α, β-unsaturated ketone. Although the Michael-type addition of sulfhydryl group to α, β-unsaturated compounds has been reported in some papers[4], it is interesting to note that an analogous reaction can occur naturally in higher plant tissue. Pharbitic acid is contained in relatively high quantity (3.5 mg/fresh weight kg of Pharbitis seeds) and does not show gibberellin activity in some biological tests. Therefore, pharbitic acid, as well as gibberellin A_3-β-glucopyranoside which itself is inactive[3], seems to function physiologically as regulating the content and the activity of gibberellin A_3.

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