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CHEMICAL STUDIES ON THE CONSTITUENTS OF *PTERIS CRETICA* L.(PTERIDACEAE)

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Pteris cretica L., known as a Chinese folk-medicinal plant[1], is one of the common ferns ranging in the temperate and torrid zones, yet very little information has been available so far on the chemistry of this species[2]. The present studies deal with the structural elucidation of two new diterpenoids designated as compound A and B, isolated from the rhizome.

Compound A(I), mp. 201-203°, $[\alpha]_D^{25}$-31.6°(c=0.38, pyridine), is considered to be a diterpenoid since its molecular formula was established as $C_{20}H_{32}O_2$. The infrared(IR) spectrum of the compound reveals the presence of a hydroxyl(3400 cm^{-1}) and a terminal methylene(3060, 1660 and 890 cm^{-1}) functions, while the nuclear magnetic resonance(NMR) spectrum* shows the existence of three tertiary methyls at 0.91, 0.98 and 1.10 ppm(3H each singlet), a slightly broadened signal centered at 3.85 ppm(1H) and a complex signal at 3.95 ppm(1H), assignable respectively to a proton geminal to a secondary hydroxyl function. Furthermore, the NMR spectrum affords the additional evidence for the terminal methylene by two singlets at 5.12 and 5.25 ppm(1H each).

Treatment of I with acetic anhydride in pyridine yielded a diacetate(II), $C_{24}H_{36}O_4$, mp. 134-136°, which exhibits two sharp singlets of two acetoxyls at 2.02 and 2.07 ppm, a multiplet at 5.06 ppm(1H, $>C\underline{H}$-OAc) and another slightly broadened signal at 5.15 ppm(1H, $>C\underline{H}$-OAc), overlapped with the signal due to the terminal methylene(at 5.15 and 5.31 ppm) in its NMR spectrum.

On the other hand, oxidation of I with chromic anhydride-pyridine complex afforded a diketone(III), $C_{20}H_{28}O_2$, mp. 173-175°, as the major and a monoketone(IV), $C_{20}H_{30}O_2$, mp. 147-149°, as the minor. It is important to point out here that, in the IR spectra of III and IV, the absorption band due to $>C=CH_2$ shifts to the higher frequency of 940 cm^{-1} [3], whereas in the NMR spectra of III and IV, two protons of $>C=CH_2$ appear as two singlets at 5.31 and 6.01 ppm which are significantly deshielded presumably due to the neighboring carbonyl group. The presumption is further supported by the characteristic ultraviolet absorption spectrum of α,β-unsaturated ketone with λ_{max}^{EtOH} 235 nm(ε=7700).

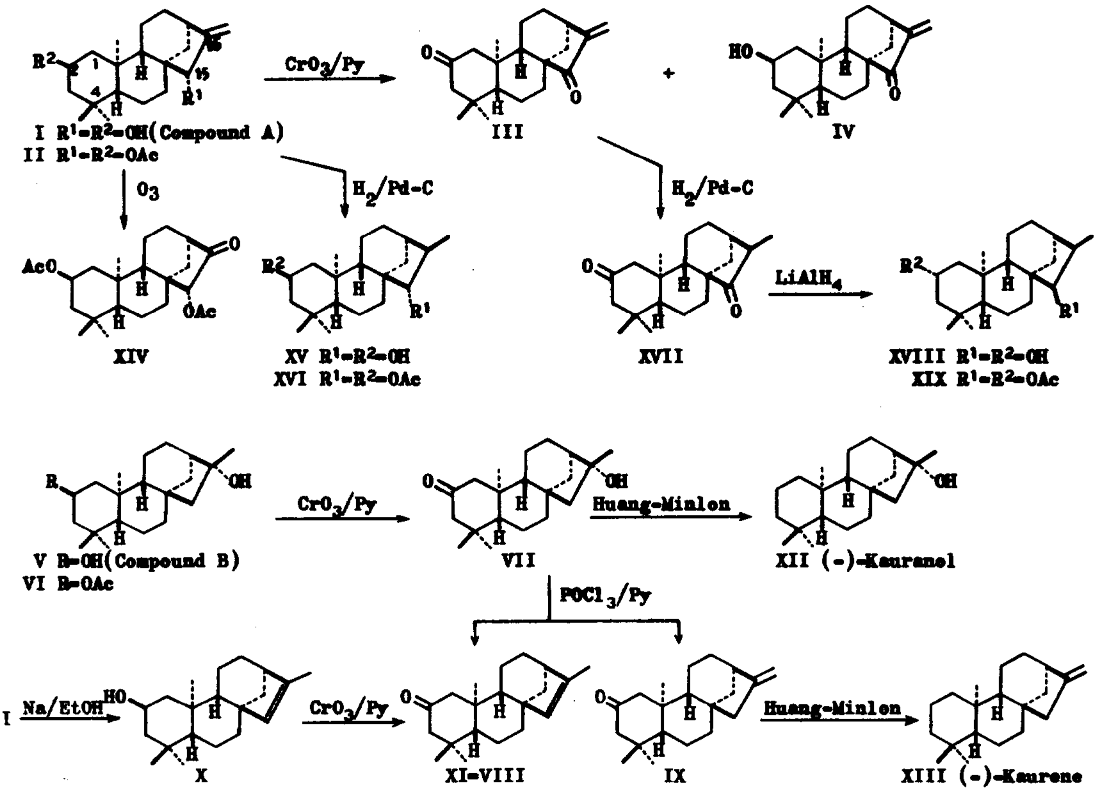
Compound A, therefore, could be assigned a tetracyclic diterpene diol of either phyllocladene- or kaurene-like skeleton, in which one of the secondary hydroxyl group is located on the adjacent carbon of the terminal methylene namely at C-15. Moreover, a multiple signal due to another $>$CH-OH of IV centered at 3.95 ppm is analyzed as a triplet of two triplets with J_{ax-ax}=11.25 c/s and J_{ax-eq}=3.75 c/s. It follows that the proton geminal to the hydroxyl group is oriented axial and couples with two axial protons and two equatorial protons located on two neighboring carbon atoms. Consequently, the second hydroxyl group of compound A is placed at C-2 with equatorial orientation, which is the only reasonable assignment in the assumed skeleton fulfilling the requirement$^{4)}$.

Compound B(V), $C_{20}H_{34}O_2$, mp. 215-218°, $[\alpha]_D^{25}$+14.4°(c=0.25, pyridine), is a diol and shows the similar spectral properties as compound A, except that the former possesses four tertiary methyls and no double bond. On acetylation of V with acetic anhydride in pyridine there was obtained a monoacetate(VI), $C_{22}H_{36}O_3$, mp. 149-151°, ν_{max}^{KBr} 3350, 1740 cm^{-1}, whose NMR spectrum shows the existence of four tertiary methyls at 0.91, 0.93, 1.13 and 1.38 ppm($-O-\overset{|}{\underset{|}{C}}-CH_3$) and a sharp singlet at 2.03 ppm(CH_3COO). In addition, a one-proton triplet of triplets at 5.13 ppm (J_{ax-ax}=11.25 c/s, J_{ax-eq}=3.75 c/s) could be assigned to a proton geminal to a secondary equatorial acetoxyl function at C-2 as in the compound A derivatives. The one hydroxyl function of V resisted both the acetylation and oxidation as described below.

Oxidation of V with chromic anhydride-pyridine complex afforded a monohydroxy-monoketone (VII), $C_{20}H_{32}O_2$, mp. 148-150°, ν_{max}^{KBr} 3300, 1715 cm^{-1} with a negative Cotton effect($[\alpha]_{310}$-1560°), Since the carbonyl group is located at C-2, the ORD evidence discloses that VII possesses the trans A/B junction with 10α-CH_3 and 5β-H as (-)-kaurene.

Compound B, therefore, is presumed to be a saturated tetracyclic diterpene carrying two hydroxyl functions, one of which is inferred a tertiary hydroxyl group probably at C-16 and the other is a secondary one with equatorial orientation at C-2. Dehydration of VII with phosphorous oxychloride in pyridine gave a mixture of two isomeric unsaturated ketones which were separated using thick-layer chromatoplates of silica gel impregnated with silver nitrate into the major product(VIII), $C_{20}H_{30}O$, mp. 133-134°, ν_{max}^{KBr} 1715, 814 cm^{-1}, and the minor(IX), $C_{20}H_{30}O$, mp. 115-116°, ν_{max}^{KBr} 1715, 880 cm^{-1} of having a terminal methylene function.

On the other hand, reduction of compound A(I) with sodium in ethanol$^{4)}$ gave a monohydroxy-olefin(X), $C_{20}H_{32}O$, mp. 148-150°, ν_{max}^{KBr} 3350, 814 cm^{-1}. Subsequent oxidation of X with chromic anhydride-pyridine complex gave a ketone(XI), which was proved to be identical in all respects



(TLC, mixed mp., IR and NMR) with the above ketone(VIII) derived from compound B. By virtue of the correlation mentioned above, it has become evident that both compounds A and B possess the same carbon skeleton.

On reduction under Huang-Minlon condition, VII gave a monool(XII), $C_{20}H_{34}O$, mp. 208-210°, $[\alpha]_D^{25}$ -44.3°(c=0.21, $CHCl_3$). Likewise, IX gave a hydrocarbon(XIII), $C_{20}H_{32}$, mp. 51°, $[\alpha]_D^{25}$-66° (c=0.11, $CHCl_3$). XII and XIII thus obtained were identified in every respects(TLC, mixed mp., IR and NMR) with (-)-kauranol[5] and (-)-kaurene[6], respectively. Furthermore, ozonolysis of the diacetate of compound A(II) gave a norketone(XIV), $C_{23}H_{34}O_5$, mp. 123-126°, ν_{max}^{KBr} 1760, 1745 cm^{-1}, which exhibited a positive Cotton effect($[\alpha]_{340}$ +2676°) indicating the β,β-configuration of the C-15 and C-16 linkage.

Accordingly both compounds A and B have now been elucidated as having the absolute configuration of (-)-kaurene and moreover, it has become clear that the tertiary hydroxyl group at C-16 of compound B(V) has the α-configuration as in (-)-kauranol(XII).

Finally, our attention was focussed to establish the configuration of the hydroxyl group at

C-15 of compound A. The fact that compound A(I), on treatment with methanolic hydrogen chloride, did not undergo the allylic rearrangement to the corresponding C-15 ketone$^{7)}$, leads us to put forward the 15α-OH configuration as most probable. Catalytic hydrogenation of compound A(I) afforded a dihydro-derivative(XV), $C_{20}H_{34}O_2$, mp. 212-215°, with 16β-CH_3 configuration$^{4,6)}$ as the major product. In the NMR spectrum of the diacetate(XVI), $C_{24}H_{38}O_4$, mp. 113-115°, the proton at C-15 appeared as a doublet centered at 4.60 ppm with J=3.5 c/s. Catalytic hydrogenation of the diketone(III), on the other hand, gave a saturated ketone(XVII), $C_{20}H_{30}O_2$, mp. 199-202°, which in turn was treated with lithium aluminum hydride to afford a diol(XVIII), $C_{20}H_{34}O_2$, mp. 132-135° possessing 2α-OH$^{4)}$ and 15β-OH group$^{8)}$. The NMR spectrum of the diacetate(XIX), $C_{24}H_{38}O_4$, mp. 74-77°, exhibited a proton at C-15 as a doublet centered at 4.76 ppm with J=11 c/s. The comparison of these NMR data of XVI and XIX has clarified that the configuration of 15-OH of XV is inverted in respect to XVIII, thus proving the α-configuration of the C-15 hydroxyl group in compound A.

In conclusion, the chemical structures including absolute configurations of compounds A and B are now established as 2β,15α-dihydroxy-(-)-kaur-16-ene(I) and 2β,16α-dihydroxy-(-)-kaurane(V) respectively.

It seems to be of interest to mention from the chemotaxonomical view-point that the present paper offers the first occurrence of (-)-kaurenoids in fern although gibberellic acid and its related compounds have already been isolated from ferns$^{9)}$.

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* Unless mentioned otherwise, the NMR spectra were determined in $CDCl_3$ solution on a JNM-4H-100 instrument, and the signals were designated in δ value using TMS as an internal standard.

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