carboxylic acid and the structure of BI-2 (I) was firmly established as the formula (E). Since the other five compounds (II—VI) were correlated each other, the structures were formulated as shown in Chart 1.

As for the absolute configuration at C-2 Hikino, *et al.*[2] proposed *R* on the basis of the CD curve. The absolute configuration of the other compounds will be reported in a forthcoming paper.

Hikino, *et al.*[3] are due to report the structures of two new glucosides named pteroside A and C from the same source as (G') and (H'). Since the aglycone (G) of pteroside A was derived to the aglycone (A) of pteroside B, the aglycone (G) is assumed to be identical with our BK-3 (VI).[4]

The coexistence of C_{14} compounds (I—III) with C_{15} compounds (IV—VI) suggests that the formers are formed from the latters and they are assumed to be formed from a common precursor like (I) or (J) derived from humulene. The presence of 1-indanones (I—VI) in ethanolic extract of fresh leaves without drying has been confirmed by thin-layer chromatography. Further separation and chemical examination along with toxicity tests are in progress in our laboratory with the colaboration of pathological laboratories.

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3) H. Hikino, T. Takahashi, and T. Takemoto, Abstracts of Papers, Annual Meeting of Pharmaceutical Society of Japan, Fukuoka, 1971, p. 777.
4) Note Added in Proof (July 20, 1971): The identity has now been confirmed by the direct comparison of the spectral data.

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Structures of Creticosides A and B, Two New Diterpenoid Glucosides from *Pteris cretica* L.

In the previous communication[1] we reported the isolation and structures of two new diterpenes, 2β,15α-dihydroxy-(−)-kaur-16-ene(I)(compound A) and 2β,16α-dihydroxy-(−)-kaurane(II)(compound B) from the rhizome of *Pteris cretica* L. (Pteridaceae). Further examination of the rhizome has enabled us to isolate two new diterpenoid glucosides and it has been shown in the present communication that these glucosides now named creticosides A and B possess the structures III and VII respectively.

A crude glycoside mixture containing creticosides A and B was obtained by column chromatography of the ether extract of the rhizome. Subsequent separation of the mixture was achieved by repeated thick-layer chromatography using the plates of silica gel impregnated with silver nitrate. Creticoside A (III), colorless needles (from acetone), $C_{26}H_{42}O_7$,

1) C.M. Chen and T. Murakami, *Tetrahedron Letters*, **1971**, 1121.

mp 179—182°, $[\alpha]_D^{18}$ −32.5° (*c*, 0.2 in pyridine), shows in the infrared (IR) spectrum (KBr) the presence of many hydroxyl groups by the strong absorption bands at 3350, 1070 and 1020 cm^{-1} and a terminal methylene group at 1660 and 890 cm^{-1}, the latter being reminiscent of the spectral characteristic of compound A (I). Acetylation of creticoside A (III) with acetic anhydride in pyridine yielded a pentaacetate (IV), $C_{36}H_{52}O_{12}$, mp 220—223°, IR ν_{max}^{KBr} cm^{-1}: 1755, 1230 (OAc), 1665, 900 (>C=CH_2), whose nuclear magnetic resonance (NMR) spectrum[2] (Table I, II) shows the existence of three tertiary methyls at 0.84, 0.91 and 1.06 ppm (3H each singlet), five acetoxyls at 1.98—2.05 ppm and a terminal methylene at 5.12 and 5.24 ppm (1H each), overlapped with the signals due to the carbinyl hydrogens at 4.83—5.30 ppm. The prominent signals at 3.6—5.3 ppm suggest the presence of sugar moiety. Based on the above observations, creticoside A (III) has been presumed to be a glycoside of compound A (I).

Enzymatic hydrolysis of creticoside A (III) using β-glucosidase was then carried out to give compound A (I), mp 201—203°, $[\alpha]_D^{18}$ −31° (*c*, 0.16 in pyridine)[1] and D-glucose. Accordingly, creticoside A (III) has now been elucidated as a glucoside of compound A (I).

The comparison of the methyl chemical shifts in the NMR spectra of compound A diacetate (Ia)[1] and creticoside A pentaacetate (IV) reveals that the methyl proton signals of IV are shifted upfield than the corresponding those of Ia[3] (Table I). This finding suggests the glucose moiety to link with compound A at C-2 rather than C-15.[4] The assumption was verified by the results shown below.

Oxidation of creticoside A (III) with chromic anhydride–pyridine complex[5] afforded a ketone (V) as the major product, $C_{26}H_{40}O_7$, mp 122—125°, IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1725 (C=O), 1648, 930 (>C=CH_2), whose ultraviolet absorption spectrum λ_{max}^{EtOH} 235 nm (ε=8100)

Table I. Methyl Chemical Shifts[2]

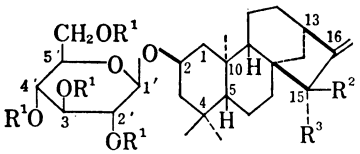
	C-4 *gem*-dimethyl	C-10 methyl	C-16 methyl
Ia	0.91, 0.94	1.14	
IV	0.84, 0.91	1.06	
IIa	0.91, 0.93	1.13	1.38
VIII	0.83, 0.90	1.04	1.34

Table II. Proton Signals[2,a]

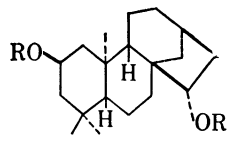
	C-2α	C-13	C-15β	>C=CH_2	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
Ia	5.06 tt	2.81 m	5.15 *	5.15, 5.31 s s						
IV	3.75 *	2.77 m	5.09 *	5.12, 5.24 * *	4.59 d	4.92 dd	5.21 *	5.03 *	3.75 *	4.17 *
VI	3.75 *	3.02 m		5.23, 5.93 s s	4.59 d	4.92 dd	5.20 dd	5.03 dd	3.75 *	4.17 *
VIII	3.75 *				4.57 d	4.91 dd	5.20 dd	5.03 dd	3.75 *	4.17 *

a) abbreviations: s=singlet, d=doublet, m=multiplet, dd=doublet of doublets, tt=triplet of triplets, *=patterns are unclear

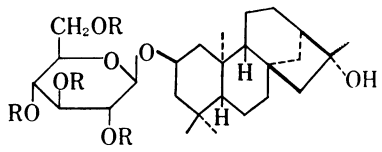
2) The NMR spectra were determined in $CDCl_3$ solution on a JNM-4H-100 instrument, and the signals were designated in δ value using tetramethylsilane (TMS) as an internal standard.
3) H. Hikino, S. Arihara, and T. Takemoto, *Tetrahedron*, **25**, 3909 (1969).
4) Although the further extensive study should be made, the present finding might be of use as a diagnostic method for the location of sugar moiety.
5) O. Theander, "Advances in Carbohydrate Chemistry," Vol. 17, Academic Press, New York, 1962, p. 264.



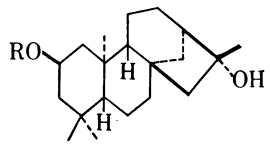
III : $R^1=R^2=H$, $R^3=OH$
(creticoside A)
IV : $R^1=Ac$, $R^2=H$, $R^3=OAc$
V : $R^1=H$, R^2, $R^3=O$
VI : $R^1=Ac$, R^2, $R^3=O$



I : R=H (compound A)
Ia : R=Ac



VII : R=H (creticoside B)
VIII : R=Ac



II : R=H (compound B)
IIa : R=Ac

Chart 1

is characterized as an α,β-unsaturated ketone. In the NMR spectrum of its tetraacetate (VI), $C_{34}H_{48}O_{11}$, mp 207—210° two protons of the terminal methylene appear as two singlets at 5.23 and 5.93 ppm which are significantly deshielded due to the neighboring carbonyl function. The combined evidence leads to the conclusion that in creticoside A the aglycone is bonded with glucose through the C-2 oxygen function as in III.

Creticoside B (VII), colorless needles (from acetone), $C_{26}H_{44}O_7$, mp 258—261°, $[\alpha]_D^{18}$ −36.3° (*c*, 0.15 in pyridine), exhibits the similar spectral properties as creticoside A. On acetylation of VII with acetic anhydride in pyridine there was obtained a tetraacetate (VIII), $C_{34}H_{52}O_{11}$, mp 226—230°, IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1755, 1230 (OAc), whose NMR spectrum shows the existence of four tertiary methyls at 0.83, 0.90, 1.04 and 1.34 ppm, the latter due to a methyl being geminal to an oxygen function, and four acetoxyls at 1.98—2.06 ppm. In addition, the signals originating from the carbinyl hydrogens of sugar moiety are observed at 3.6—5.3 ppm (Table II).

Enzymatic hydrolysis of creticoside B (VII) using β-glucosidase was also carried out to give compound B (II), mp 215—218°, $[\alpha]_D^{19}$ +13.1° (*c*, 0.12 in pyridine)[1] and D-glucose, establishing that creticoside B (VII) is a glucoside of compound B (II). Moreover, since both the IR and NMR spectra of VIII disclose the existence of the tertiary hydroxyl group at C-16, it has become clear that in creticoside B the aglycone is bonded with glucose through the C-2 oxygen function as in VII.

Furthermore, based on the fact that creticosides A and B were hydrolyzed smoothly by β-glucosidase, the glucose residue is inferred to be present as a β-anomer. This is supported by the NMR spectra of IV and VIII showing the doublets at 4.59 (1H, J=7.5 cps) and 4.57 ppm (1H, J=7.5 cps) due to the C-1′ anomeric hydrogens which indicate the β-orientation of the glucosidic linkage.[6]

6) *a*) L.M. Jackman, "Fortschritte der Chemie Organischer Naturstoffe," Vol. 23, Springer-Verlag, Wien, 1965, p. 341; *b*) I. Yosioka, M. Fujio, M. Osamura, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 6303.

Creticosides A and B are rare examples of the naturally occurring diterpenoid glycosides and their occurrence in fern is of quite interest.

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Recherches Toxicologiques des Substances Métaboliques du *Fusarium nivale*. VIII[1]. La Quatriéme Substance Métabolique de *F. nivale*

Dans une publication antérieure, nous avons déjà mentionné que trois toxines scirpenoides jouent le principal rôle dans la lésion radiomimétique provoquée aux animaux expérimentaux[1-3].

Notre équipe travaillant sur l'aspect toxicologique des micotoxines, se charge maintenant de réexaminer toutes les substances qui sont contenues dans la toxine brute. Jusqu'à present, l'on rend responsable des lésions provoquées aux animaux à qui sont administrés de la toxine brute, les substances sciprenoïdes; mais nous pensons qu'il y ait d'autres substances jouant un petit rôle dans le *fusarium*-toxicose, bien que la toxicite de ces substances soit faible.

Comme premiére revision, nous avons commencé à examiner la substance f. 127° accompagée toujours à nivalenol-4-O-acétate.

Récemment nous avons trouvé une souch qui produisait une quantité suffisante de cette substance à partir des souches concervées, et maintenant nous avons recherché la structure chimique de cette substance.

Culture et Isolement

On inocule *F. nivale*, souche Fn-2-B conservée, dans une solution de Czapek additionée de 1% de peptone et on le cultive à 25° pendant deux semaines. On ajoute 20 g/litre de charbon actif dans 25 litre de la solution de culture pour absorber les toxines, que l'on sépare ensuite du charbon en ajoutant 5 litres méthanol. On fait dissourdre la fraction libérée dans une partie de méthanol à la quelle on a ajouté 9 parties de chloroforme. Après avoir séparé le précipité, on obtient 8,2 g de la fraction soluble du mélange méthanol–chloroforme.

On chromatographie cette fraction sur une colonne de gel de silice en utilisant comme solvant un mélange chloroforme–méthanol (10:1).

Après la fraction qui contient nivalenol-4-O-acétate, nous obtenons la substance qui s'est cristallisée par le chauffage avec benzène.

Examen de la Structure Chimique

La structure chimique de cette nouvelle substance a été supposée comme étant l'anhydrid de la Prolyl–tyrosine en considérant ses propriétés physico–chimiques, spectroscopiques

1) Part VII: T. Tatsuno, Y. Morita, H. Tsunoda, and M. Umeda, *Chem. Pharm. Bull.* (Tokyo), **18**, 1485 (1970).
2) T. Tatsuno, M. Saito, M. Enomoto, and H. Tsunoda, *Chem. Pharm. Bull.* (Tokyo), **16**, 2519 (1968).
3) T. Tatsuno, Y. Fujimoto, and Y. Morita, *Tetrahedron Letters*, **1969**, 2823.