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Steroid Saponins of Aerial Parts of *Paris tetraphylla* A. Gray and of Underground Parts of *Trillium tschonoskii* Maxim.

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In contrast to the dried rhizomes of *Paris polyphylla* Sm., the dried aerial parts of *Paris tetrphylla* A. Gray contained predominantly pennogenin rhamnosyl-chacotrioside (I). From the methanol extracts of fresh underground parts of *Trillium tschonoskii* Maxim., dioscin (III) and methyl proto-dioscin (IV) were isolated and any pennogenin glycosides and the related compounds, which were found in *Trillium kamtschaticum*, could not be obtained. The ratio of yields of III to IV from the dried materials was reverse to that from the fresh ones, and the homogenate of the fresh materials was extracted only to give III. The results may support the claim that III is considered to be a secondary glycoside.

Previously it was reported[2,3a] that the dried rhizomes of *Paris polyphylla* Sm. contained three diosgenin- and one pregnane glycosides, and that from the fresh underground parts of *Trillium kamtschaticum* Pall. were obtained eight steroid saponins including pennogenin 3-O-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (rhamnosyl-chacotrioside) (I). In connection with these works, the steroid saponins in the title plants, which have close taxonomical relations to the above species but had not been investigated so far in regard to their ingredients, were surveyed.

The methanol extracts of cut and air-dried aerial parts of *Paris tetraphylla* A. Gray were shown to contain one predominant glycoside accompanied with trace amounts of four minor ones. The major glycoside was easily isolated in a pure form and identified with I which was found in *T. kamtschaticum*[3a] and also in the fresh whole plants of *Heloniopsis orientalis* (Thubn.) C. Tanaka[4] together with the corresponding 3,26-O-bisglycoside (II).

The predominant existence of I suggests that, similarly to the case of *H. orientalis*,[4] the fresh plant may contain II, which could be regarded[3] as Marker's "nolonin." The constituents of aerial parts of *P. tetraphylla* seem to be quite different from those of underground parts of *P. polyphylla*.[2]

The fresh underground parts of *Trillium tschonoskii* Maxim. were treated as shown in Chart 1 to give two major components which were identified as dioscin (III)[5] and methyl proto-dioscin (IV).[6] IV is regarded as an artefact produced from the originally existing 22-hydroxy analog, proto-dioscin (V), during the procedures of extraction and crystallization using methanol.[6] In contrast to the case of *T. kamtschaticum*,[3a] neither pennogenin glycosides

1) Location: *3-1-1, Maedashi, Higashi-ku, Fukuoka, 812, Japan.*
2) T. Nohara, H. Yabuta, M. Suenobu, R. Hida, K. Miyahara, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **21**, 1240 (1973).
3) *a*) T. Nohara, K. Miyahara, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 872 (1975); *b*) *Idem, ibid.*, **22**, 1772 (1974).
4) T. Nohara, Y. Ogata, M. Aritome, K. Miyahara, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 925 (1975).
5) T. Kawasaki and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **10**, 703 (1962).
6) T. Kawasaki, T. Komori, K. Miyahara, T. Nohara, I. Hosokawa, and K. Mihashi, *Chem. Pharm. Bull.* (Tokyo), **22**, 2164 (1974).

MeOH extractives (101 g) of fresh underground parts (1.4 kg) of *Trillium tschonoskii*

shaken with a mixture of water (600 ml) and BuOH-AcOEt (1: 4, v/v, 500 ml)

org. layer

1) evapd. to yellow syrup (21.1 g)
2) exted. with hot AcOEt (200 ml)

AcOEt solution

insoluble portion

chromatog. over silica gel (210 g) solv., CH_2Cl_2–MeOH–water (7: 3: 0.1, v/v)

Fr. 1

III

Fr. 2

IV

aq. layer

exted. with BuOH

aq. layer

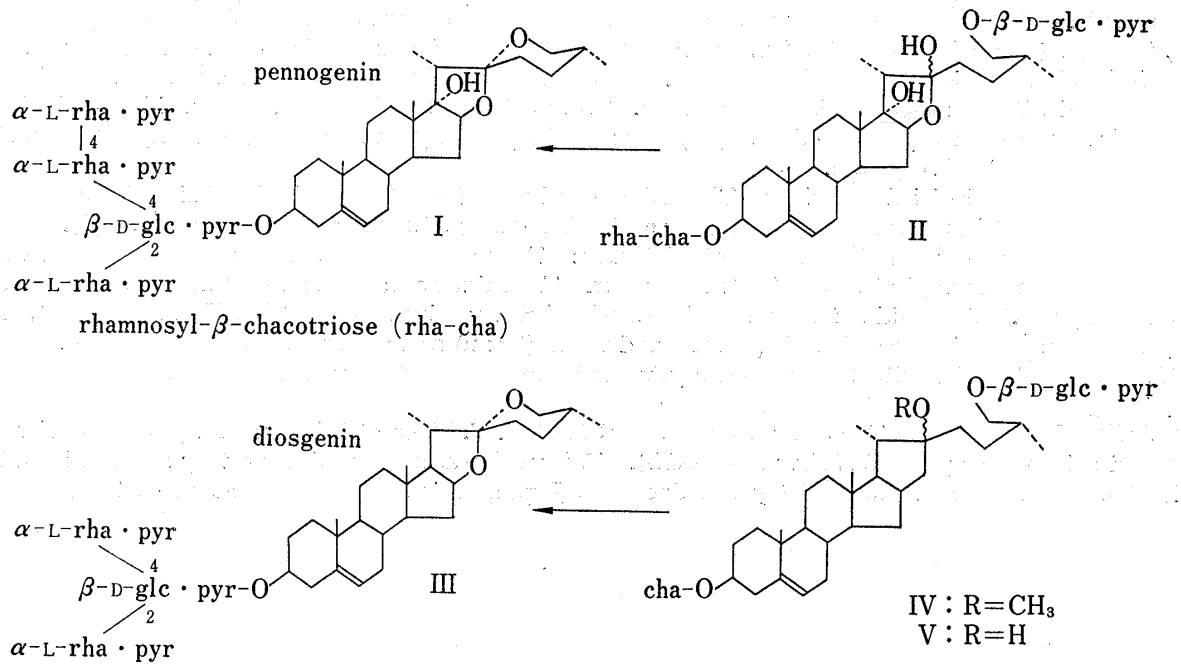
BuOH layer

evapd.

Fr. 3

IV

Chart 1



Formulae

nor the related compounds could be obtained. When the materials were sliced, air-dried and then treated in the same way, the ratio of yields of III to IV was 9.3: 1, being reverse to that (1: 9.1) in the above experiment. Furthermore, the homogenate of the fresh underground parts was extracted with methanol solely to give III.[7] These results may support the previous claim[6] that III is considered to be a secondary glycoside formed in the plants enzymatically from V.

7) According to Joly, *et al.*, V was incubated with the homogenate of the fresh leaves of *Dioscorea floribunda* to yield III (R.A. Joly, J. Bouner, R.D. Bennett, and E. Heftmann, *Phytochemistry*, **8**, 1445 (1969)).

Experimental[8]

Isolation and Identification of Major Steroid Saponin in Air-dried Aerial Parts of *Paris tetraphylla*—— The materials collected during April in the suburbs of Fukuoka city were cut and air-dried for 1 week. The dried plants (260 g) were extracted with hot MeOH (2 liters) for 30 hr. The MeOH extractives (36.2 g), which showed five spots on a thin-layer chromatogram (TLC), *Rf* 0.81 (trace), 0.63 (trace), 0.56 (trace), 0.48 (major), 0.22 (trace), all being negative to the Ehrlich test and positive (orange yellow) to dil. H_2SO_4 test, were shaken with BuOH (250 ml) and water (150 ml). The organic layer was evaporated *in vacuo* to a brown syrup (12.6 g) which was defatted with hot hexane. The residue (5.1 g) was chromatographed over silica gel and the eluate with CH_2Cl_2–MeOH–water (8: 2: 0.1, v/v) was evaporated to dryness and crystallized from MeOH to give the major glycoside (*Rf* 0.48) as colorless needles (210 mg), mp 223—227° (decomp.), $[\alpha]_D^{28}$ —140.5° (c=1.3, pyridine). It was hydrolyzed with HCl in MeOH to give the same products as from pennogenin glycosides (Tb, Tc, Tg (I)) of *T. kamtschaticum*,[3a] and identified with an authentic sample (Tg) of pennogenin 3-O-rhamnosyl-chacotrioside (I) by mixed mp and by comparisons of their *Rf* values on TLC and infrared (IR) spectra.

Isolation and Identification of Major Steroid Saponin(s) in Underground Parts of *Trillium tschonoskii*—— a) From the Fresh Materials: The underground parts (1.4 kg) which were collected during May in Chitose (Hokkaido) and packed in sawdust were, after 10 days, sliced and immediately soaked in cold MeOH (1.5 liters) for 2 weeks. The extractives (101 g) showed four spots on TLC, *Rf* 0.63 (Ehrlich —),[9] 0.56 (Ehrlich —) (major), 0.33 (Ehrlich +) (major), 0.25 (Ehrlich +),[10] all being positive (orange yellow) to dil.H_2SO_4 test. They were treated as shown in Chart 1. Fraction 1 gave on crystallization from MeOH the compound of *Rf* 0.56 (Ehrlich —) as colorless needles (2.4 g, 0.17%), mp 276—279° (decomp.), $[\alpha]_D^{25}$ —108.8° (c=1.21, EtOH), 25D-spiroketal absorptions on the IR spectrum. It was hydrolyzed with acid to provide diosgenin, glucose and rhamnose, and identified as dioscin (III) by direct comparison (mixed mp, *Rf*, IR) with an authentic sample.[5] Fractions 2 and 3 were combined and crystallized from MeOH to give the compound of *Rf* 0.33 (Ehrlich +) as colorless needles (21.7 g, 1.55%), mp 188—191° (decomp.), $[\alpha]_D^{25}$ —92.9° (c=0.92, pyridine), no spiroketal absorptions on the IR spectrum. It was hydrolyzed with acid to give the same products as from III and with almond emulsin (Sigma Chem. Co.) to afford III and glucose. It was identified with an authentic sample of methyl proto-dioscin (IV)[6] by mixed mp and by comparisons of their *Rf* values on TLC, IR and NMR (in C_5D_5N) spectra.

b) From the Air-dried Materials: The underground parts (250 g) were sliced and air-dried for 2 weeks. They were treated in the same way as above to give III (2.55 g, 1.02%) and IV (0.28 g, 0.11%).

c) From the Homogenate of the Fresh Materials: The underground parts (90 g) were sliced, homogenized and extracted with hot MeOH (200 ml) for 3 hr. The mixture was filtered and the filtrate was evaporated to dryness. The residue (6.8 g), showing two Ehrlich negative spots on TLC (*Rf* 0.56 (major), 0.63), was shaken with a mixture of BuOH (100 ml) and water (80 ml) and the BuOH layer was evaporated to a solid. It was defatted with hexane and crystallized from MeOH to give III (0.85 g).

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8) Melting points were determined on a Kofler block and are uncorrected. Optical rotations were taken with a JASCO DIP-SL automatic polarimeter, and infrared spectra were obtained with a JASCO IR-G spectrometer. Thin-layer chromatography was performed on Kieselgel G nach Stahl (Merck) using $CHCl_3$–MeOH–water (7: 3: 0.5, v/v) as solvent. Column chromatography was carried out with "Kanto" silica gel (100—200 mesh).

9) Likely to be a prosapogenin of dioscin (*cf.* ref. 3. 6).

10) Most likely to be V (*cf.* ref. 6).