Tabelle 1. ^{1}H-NMR-Signale von 3 (270 MHz, TMS als innerer Standard, δ-Werte)

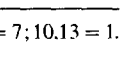
	$CDCl_3$, 270 MHz	+ $Eu(fod)_3$
2-H	*dd* 1.54	*dd* 1.54
3-H	*dd* 0.66	*dd* 0.67
3'-H	*dd* 0.75	*dd* 0.76
5-H	*m* 1.25	*m* 1.26
6-H	*m* 2.00	*m* 2.03
7-H	*m* 1.20	*m* 1.22
8-H	*m* 1.34	*m* 1.35
9-H	*m* 2.10	*m* 2.18
10-H	*tq* 5.45	*t(br)* 5.64
12-H	*s(br)* 4.45	*s(br)* 5.16
13-H	*s(br)* 1.66	*s(br)* 1.85
14-H	*d* 0.96	*d* 0.97
15-H	*s(br)* 4.63	*s(br)* 4.63
15'-H	*s(br)* 4.81	*s(br)* 4.81
OAc	*s* 2.08	*s* 2.73

J(Hz): 2,3 = 8; 2,3' = 3.5; 3.3' = 4; 7.14 = 6.5; 9,10 = 7; 10.13 = 1.

läßt sich auch die Konfiguration der trisubstituierten Doppelbindung bestimmen. Obwohl nicht alle Signale interpretierbar sind, läßt sich durch Doppelresonanzexperimente zeigen, daß ein Cyclopropanring vorhanden sein muß, der trisubstituiert ist. Während die Protonen an C-3 Signale bei sehr hohem Feld zeigen (*dd(br)* 0.65 und *dd* 0.75), liegt das Signal des dritten Dreiringprotons, wie Doppelresonanz-Experimente zeigen, als *dd* bei 1.54. Dieses ist nur mit einer allylischen Stellung dieses Protons vereinbar. Nach Zusatz von $Eu(fod)_3$ werden die sonst zusammenfallenden Signale der allylischen Protonen getrennt, und man kann durch Entkopplung zeigen, daß neben der exocyclischen Doppelbindung eine CH_2-Gruppe steht, die mit einem CH_2-Multiplett bei *ca* 1.20 koppelt. Die Zuordnung der übrigen Signale läßt sich ebenfalls durch Entkopplungen sichern. Auch das Massenspektrum ist gut mit der Konstitution 3 vereinbar. Besonders deutlich ist die Abspaltung der Seitenkette unter Bildung eines Fragmentes C_7H_9 zu beobachten. Offen bleibt jedoch die Konfiguration an C-2, C-4 und C-7. Der Grundkohlenwasserstoff ist das bereits bekannte Sesquisabinen [3].

EXPERIMENTELLES

100 g Wurzeln von *Arctotis grandis* Thunb. (angezogen aus Samen vom Botanischen Garten Chelsea, Herbar Nr. 77/965) wurden frisch zerkleinert und mit Et_2O/Petrol 1:2 bei Raumtemp. extrahiert. Der Extrakt ergab nach DC (Sigel, GF 254) 1 mg **2**, Spuren von **1** und 16 mg **3** (Et_2O/Petrol 1:10).

12-*Acetoxy-sesquisabinen* (**3**): Farbloses Öl. IR: OAc 1745, 1230; C=C 3080, 1650, 870 cm^{-1}. MS: M^+ *m/e* 262.193 ($C_{17}H_{26}O_2$) (6%), —AcOH 202 (8); 202 —*Me 187 (9);  93 (60); $H_3C\,CO^+$ 43 (100).

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546\ nm}{-36.9 \quad -38.5 \quad -43.6°} \quad (c = 1.57, CHCl_3).$$

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SENKIRKINE AND A NEW SESQUITERPENE GLYCOSIDE, ISOPETASOSIDE, FROM *PETASITES JAPONICUS*

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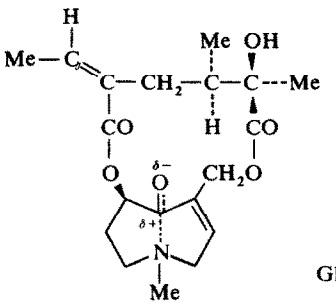
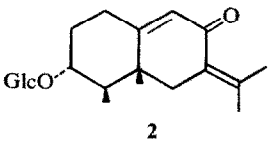
Key Word Index—*Petasites japonicus*; Compositae; pyrrolizidine alkaloids; senkirkine; sesquiterpene glycoside; isopetasoside; isopetasol.

Petasites japonicus, which is used both as a food and herbal remedy, has been previously examined for sesquiterpenes [1–3], phenolics [4], and essential oils [5]. Carcinogenicity of the young flower stalks of this plant was reported by one of us (I.H.) [6] and in a search for the carcinogen(s), we isolated two new pyrrolizidine alkaloids, petasitenine and neopetasitenine, the structures of which have been elucidated [7, 8]. Furuya *et al.* [9] also isolated a pyrrolizidine alkaloid which they called fukinotoxin [9], but which in fact is petasitenine [10]. Petasitenine has recently been proved to be a carcinogen [11]. Further scrutiny of the alkaloids of *P. japonicus* has now resulted in the isolation of senkirkine (**1**) [12], a minor alkaloid (the highest yield obtained: *ca* 0.0001% based on the weight of the dried plant material), which may also be responsible for the carcinogenicity of this plant. The amount of **1** varied with plant age and there was more in the rhizomes of the mature plant (e.g. 0.0026% based on dry wt.) than in the young flower stalks.

A new sesquiterpene glycoside, isopetasoside (**2**) was isolated and characterized as its tetraacetate. Based on spectral properties, the aglycone of **2** was deduced to be isopetasol [13–15]. Acid hydrolysis of **2** afforded glucose and isopetasol. Assignment of the β-anomeric configuration of the glucopyranoside moiety in **2** was based on the



1



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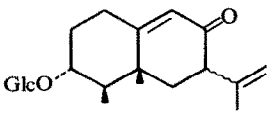

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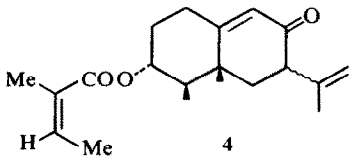

4

PMR spectral evidence: a doublet due to the anomeric proton in the PMR spectrum of the acetate appeared at δ 4.62 with the coupling constant of 7.5 Hz. The D-configuration of glucose was determined by enzymatic means. Thus isopetasoside is (+)-isopetasol-β-D-glucopyranoside (**2**). It should be noted that isopetasoside (**2**) was isolated as an inseparable mixture together with a closely related glucoside (**3**), the presence of which was apparent from the PMR spectroscopy. On treatment with base (NaOMe–MeOH, room temp.) the mixture of **2** and **3** was converted to pure **2**. There is thus the possibility that the double bond of the isopropenyl group in **3** isomerized in the course of extraction to produce **2**; such ready isomerization is known to occur during the hydrolysis of petasin (**4**) to isopetasol [14].

EXPERIMENTAL

Mps were uncorrected. IR: JASCO Model IRS, $CHCl_3$. PMR: Varian HA-100 (100 MHz), δ-values, TMS as internal standard. MS: Hitachi RMU-6C, direct inlet system, 70 eV. Optical rotations: JASCO Model DIP-SL. For TLC, Si gel GF_{254}, PF_{254} and alumina GF_{254}, PF_{254}-Type T (Merck) were used. Organic solns were dried over Na_2SO_4.

Isolation of senkirkine (**1**). Young flower stalks of *Petasites japonicus* were collected in late March and early April in Kawai, Niu, and Shokawa Villages of Gifu Prefecture (Japan), dried at room temp. and ground. The powdered material (53 kg) was extracted in EtOH at room temp. for 10 days. After filtration, the concd soln (*ca* 10 l.) was acidified (pH 2) with 6N H_2SO_4, and the mixture was exhaust. extracted with Et_2O. The aq. phase was made basic (pH 8) with conc NH_4OH, and exhaustively extracted with $CHCl_3$. The $CHCl_3$ extracts were evapd giving a dark brown residue, which was chromatographed on Si gel (Merck, No. 7734) with MeOH. The alkaloidal fractions were concd, giving a yellow resinous material, which was chromatographed on Al_2O_3 (Merck, No. 1097) with $CHCl_3$. Further separation by PLC on Al_2O_3 with EtOAc gave senkirkine (**1**) (50 mg), mp 192–195° (EtOAc), $[\alpha]_D^{19}$ −11° (MeOH; c = 0.82), (lit. [12] mp 196.5–197.5°, $[\alpha]_D^{25}$ −16° (MeOH: c = 1.89)). (Found: C, 62.35; H, 7.35; N, 3.93. Calc. for $C_{19}H_{27}O_6N$: C, 62.45; H, 7.45; N, 3.83%). Identification with authentic **1** was performed by spectral (IR, PMR, MS) and TLC (Al_2O_3, EtOAc) comparison.

Isolation of isopetasoside (**2**). Dried and ground young flower stalks of *P. japonicus* (200 g) were extracted in EtOH (2 litre) at 50° for 7 hr. After filtration, the filtrate was concd. and the residue diluted with H_2O (100 ml) and washed with Et_2O (3 × 100 ml). The aq. phase was extracted with $CHCl_3$ (3 × 150 ml) and the combined extracts concd. The oily residue (370 mg) was purified by PLC on Si gel (MeOH–$CHCl_3$, 1:4) to give a mixture of **2** and **3** (72 mg) as a pale yellow oil. A mixture of **2** and **3** (35 mg) was acetylated with Ac_2O–Py ,and the product purified by PLC on Si gel (EtOAc–$CHCl_3$, 1:2) to give a crystalline mixture of the two acetates (25 mg) in the ratio of 6:4. To a soln of the two acetates (14 mg) in MeOH (0.5 ml) was added a soln (1.5 ml) of NaOMe in MeOH (Na (100 mg) and MeOH (10 ml)) and the mixture stirred at room temp. for 40 min, neutralized by ion exchange resin (Amberlite CG-50), and filtered. The concd filtrate was purified by PLC on Si gel (EtOAc–$CHCl_3$, 1:1) yielding pure **2** (6.5 mg) as a colourless oil; yield 0.012%; $[\alpha]_D^{20}$ +7.3° (EtOH; c = 2.2); IR ν_{max} cm^{-1}: 3375, 1652, 1624, 1610 (*sh*); PMR ($CDCl_3$) 0.98 (3H, *s*), 1.06 (3H, *d*, J = 6.0 Hz), 1.84 (3H, *s*), 2.09 (3H, *s*), 5.76 (1H, *s*); MS *m/e*: 396 (M^+), 235, 234.

Isopetasoside tetraacetate. Isopetasoside (**2**) (6.5 mg) was acetylated with Ac_2O–Py and the product purified by PLC on Si gel (EtOAc–$CHCl_3$, 1:1) to afford the acetate (7.4 mg), mp 186–188° (*n*-hexane–C_6H_6); $[\alpha]_D^{20}$ −10.6° ($CHCl_3$; c = 1.2); IR ν_{max} cm^{-1}: 1753, 1652, 1625, 1610; PMR ($CDCl_3$) 0.98 (3H, *s*), 1.04 (3H, *d*, J = 6.0 Hz), 1.85 (3H, *br s*), 2.01 (3H, *s*), 2.03 (3H × 3, *br s*), 2.08 (3H × 2, *br s*), 3.58 (1H, *m*), 3.70 (1H, *m*), 4.27 (2H, *m*), 4.62 (1H, *d*, J = 7.5 Hz), 4.85–5.40 (3H, complex pattern), 5.76 (1H, *d*, J = 1.5 Hz); MS *m/e*: 564 (M^+). (Found. C, 61.78. H, 7.12. $C_{29}H_{40}O_{11}$ requires: C, 61.69; H, 7.14%).

Acid hydrolysis of isopetasoside (**2**). A soln of **2** (13 mg) in MeOH (1 ml) and 2.5 N HCl (1 ml) was gently refluxed for 90 min, concd and exhaustively extracted with $CHCl_3$. The combined concd $CHCl_3$ extracts were purified by PLC on Si gel (EtOAc–$CHCl_3$, 1:1) yielding isopetasol (4.5 mg), mp 124–125° (*n*-hexane–C_6H_6) (authentic sample, mp 124–125°; mixed mp 124–125°); $[\alpha]_D^{25}$ +72.5° (EtOH; c = 0.4) (lit [14], $[\alpha]_D$ +116.6° ($CHCl_3$; c = 1.04)). Identification was confirmed by IR, PMR, and MS comparison with an authentic marker. The presence of glucose in the aq. phase was proved by PC.

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