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SHORT COMMUNICATION

STEROIDS OF *CYNANCHUM GRANDIFOLIUM* VAR. *NIKOENSE**†

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Abstract—Two aglycones were isolated from *Cynanchum grandifolium* and identified as hirundigenin and anhydrohirundigenin.

WE HAVE isolated several polyhydroxypregnane homologues from the various *Cynanchum* species.[1] The present paper describes the study of these components in *C. grandifolium* var. *nikoense* (Maxim.) Ohwi, which is indigenous to Western Japan.

The dried whole plants were extracted with chloroform and processed to give a crude glycoside mixture, which was hydrolyzed under mild acidic conditions. From the sugar portion of the hydrolyzate, digitoxose, oleandrose, and cymarose were identified by PPC and TLC. The aglycone fraction was chromatographed on an alumina column, but no clear-cut fraction was obtained. Subsequently, on the presumption that it might be an inseparable mixture of ester glycosides as found in many other cases, the aglycone fraction was treated with dilute alkali. The amount of the fraction which could be recovered greatly decreased during this process. The neutral fraction from this hydrolysis afforded three crystalline compounds; β-sitosterol, substance A, m.p. 185–200° and substance B, m.p. 175–178°/205–220°.

While an effort was being made to collect more plant material for further investigation, a Swiss and a British group jointly reported the isolation of hirundigenin (I) and anhydrohirundigenin (II) from *Vincetoxicum hirundinaria*, the structures of which as novel 15-oxapregnanes were assigned by X-ray crystallography.[2]

* Part XXVII in the series Constituents of Asclepiadaceae Plants; for Part XXVI, see *Chem. Pharm. Bull. Tokyo* submitted.

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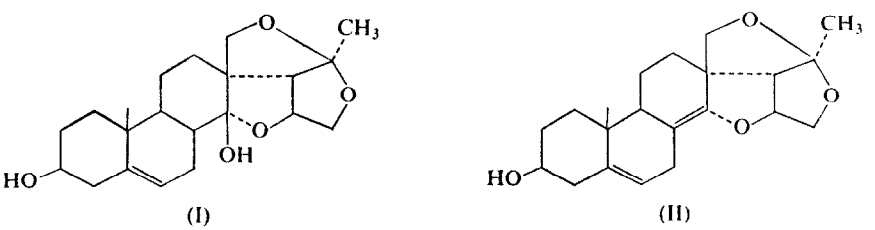
[1] H. MITSUHASHI and Y. SHIMIZU, *Chem. Pharm. Bull. Tokyo* **8**, 313 (1960); H. MITSUHASHI and Y. SHIMIZU, *Chem. Pharm. Bull. Tokyo* **10**, 719 (1962); H. MITSUHASHI, Y. SHIMIZU, T. NOMURA, T. YAMADA and E. YAMADA, *Chem. Pharm. Bull. Tokyo* **11**, 1198 (1963); Y. SHIMIZU and H. MITSUHASHI, *Tetrahedron* **24**, 4143 (1968); H. MITSUHASHI, K. SAKURAI, T. NOMURA and N. KAWAHARA, *Chem. Pharm. Bull. Tokyo* **14**, 712 (1966); H. MITSUHASHI, K. HAYASHI and T. NOMURA, *Chem. Pharm. Bull. Tokyo* **14**, 779 (1966).

[2] O. KENNARD, J. K. FAWCETT, D. G. WATSON, K. A. KERR, K. STÖCKEL, W. STÖCKLIN and T. REICHSTEIN, *Tetrahedron Letters* 3799 (1968).

Although a discrepancy was found between the melting point of substance B and that of hirundigenin, the properties of both compounds were similar and their identity was established by direct comparison. Substance A was also identified with anhydrohirundigenin by TLC. As is evident from the structure, both compounds are extremely labile in acidic media, which may explain the poor yield of the aglycones which we obtained. It also is uncertain if both compounds have the same glycosidic pattern in the original extract.



(I) (II)

FIG. 1.

EXPERIMENTAL

Extraction and Isolation of Crude Glycoside

The dried whole plant (1·6 kg) collected near Mt. Ibuki in November was extracted with $CHCl_3$ at room temp. The extract was condensed to a small volume, to which a large amount of *n*-hexane was added. The hexane layer was concentrated and treated again with *n*-hexane. Trituration of the combined precipitate gave a powdery precipitate, which was dissolved in MeOH, and, after removal of the insoluble material, was concentrated to an amorphous crude glycoside mixture (29 g).

Acid Hydrolysis of Crude Glycoside

(*a*) The crude glycoside (15 g) was dissolved in 0·05 N H_2SO_4 in 75% MeOH (200 ml) and heated under reflux for 25 min. After addition of water (150 ml), MeOH was removed *in vacuo* at 50°. The mixture was extracted with Et_2O and then with $CHCl_3$. The extract was washed with H_2O, 5% $NaHCO_3$ and H_2O, and dried over Na_2SO_4. Evaporation of the solvent gave the crude aglycone. (Yields: 5·5 g from the ether extract and 2·0 g from $CHCl_3$ extract.)

The water layer was neutralized with 5% $Ba(OH)_2$ and concentrated in the presence of a small amount of $BaCO_3$. The residue was extracted with MeOH. The MeOH solution was concentrated to a syrupy sugar fraction (7·7 g) (Keller–Kiliani reaction: positive). Examination by TLC (SiO_2 HF_{254}, 5% MeOH–$CHCl_3$) and PPC (1% NH_4OH–BuOH, ascending method, 20 hr: Formamide–$CHCl_3$, descending method, 5·5 hr) identified digitoxose, cymarose and oleandrose.

(*b*) Since the above aglycone fraction still showed positive Keller–Kiliani reaction, the Et_2O extract (4·5 g) and $CHCl_3$ extract (2·0 g) were combined and treated as the procedure (*a*). Yields: 3·5 g from the Et_2O extract, 0·7 g from $CHCl_3$ extract and 1·2 g from sugar fraction.

(*c*) The crude glycoside (13·5 g) was hydrolyzed with 0·05 N H_2SO_4 in 75% MeOH (667 ml) under reflux. After addition of water (500 ml), MeOH was removed *in vacuo*. The resulting mixture was extracted with $CHCl_3$. After the usual processing, 7 g of aglycone fraction and 6·3 g of sugar fraction were obtained.

Alkali Hydrolysis of Aglycone

The combined aglycone fractions from the experiments (*b*) and (*c*) (9 g) were dissolved in 5% methanolic KOH solution (400 ml). The solution was refluxed for 5 hr under N_2. After evaporation of MeOH *in vacuo*, the mixture was extracted continually with CH_2Cl_2 for 15 hr. Evaporation of the solvent gave an amorphous residue (2·5 g). The H_2O layer was extracted again with water-saturated BuOH. The BuOH layer was washed with BuOH-saturated H_2O. Evaporation of BuOH under a reduced pressure gave 2·7 g of residue, which was not studied further.

Chromatography of Aglycone

The CH_2Cl_2 extract (2·5 g) obtained by the above alkaline treatment was chromatographed over alumina (100 g). Fractions eluated with 0·2% MeOH in benzene gave crystals from MeOH. Recrystallization from the same solvent afforded β-sitosterol, m.p. 136·5–138·5° (37·8 mg).

Hirundigenin

Fraction eluated with 0·5% MeOH in benzene afforded a crystalline mixture, m.p. 168–173°/180–193°, which showed two major spots on TLC (alumina G, 3% MeOH in benzene; SiO_2 HF_{254}, $MeCl_2$–EtOAc 3:1). The mixture was submitted to preparative TLC (alumina G, 3% MeOH in benzene). The sample eluted from two zones proved to be the mixture of two spots and repetition gave the same result. The samples were recombined (total 20 mg) and submitted to preparative TLC (SiO_2 HF_{254}, $MeCl_2$–EtOAc, 3:1). Two zones were scraped off and extracted with Et_2O. The upper zone gave crystals, m.p. 185–200°, i.r. ν_{max} cm^{-1}: 3490, 1718, which showed two spots on TLC after several attempts, but the major spot coincided with anhydrohirundigenin (II) on TLC. The lower zone also gave a crystalline mixture, which recrystallized repeatedly from acetone to needles (7 mg), m.p. 175–178/205–220°, $[\alpha]_D$ −55·2° (C = 0·8, $CHCl_3$). (Found: C, 69·70; H, 8·39. Calc. for $C_{21}H_{30}O_5$: C, 69·58; H, 8·34.) I.r. ν_{max}^{Nujol} cm^{-1}; 3480, 1038, 987, 950, 890, 838, 802. NMR (τ in $CDCl_3$): 8·51 (3H, singlet, CH_3), which was proved to be identical with hirundigenin in all respects.

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