Isolation and Characterization of Cardiotonic Steroids from the Bulb of Urginea altissima BAKER

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Isolation and Characterization of Cardiotonic Steroids from the Bulb of *Urginea altissima* Baker

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Six cardiotonic steroids were isolated from bulbs of *Urginea altissima* Baker and identified as hellebrigenin, scilliglaucosidin, scilliglaucosidin-3-one, altoside, hellebrigenin β-D-glucoside, and scilliglaucosidin α-L-rhamnoside.

Keywords——cardiotonic steroid; *Urginea altissima* Baker; hellebrigenin β-D-glucoside; scilliglaucosidin α-L-rhamnoside; scilliglaucosidin-3-one; cytotoxicity; Straub's preparation

Altoside (IIe) is the only cardiotonic steroid hitherto isolated from *Urginea altissima* Baker,[2] although the occurrence of antimicrobial alkaloids in this plant has been reported.[3] The present paper deals with the isolation and characterization of six cardiotonic steroids from the bulb of *Urginea altissima* Baker. Bulbs collected in Kenya were extracted with ethanol and the insoluble material was reextracted with chloroform using a Soxhlet extractor. Hellebrigenin (Ia),[4] scilliglaucosidin (IIa),[3] and scilliglaucosidin-3-one (IIb)[4-6] were separated from the chloroform extract. These aglycones were unequivocally characterized by direct comparison with corresponding authentic samples. To the best of our knowledge this is the first reported isolation of scilliglaucosidin-3-one from a plant source, although the preparation of this compound from scilliglaucosidin and hellebrigenin by chemical and microbial oxidation has been reported.[4] One-seventh of the ethanolic extract was partitioned in an ethyl acetate-water system, and the three aglycones (Ia, IIa, IIb) were also obtained from the organic layer.

The aqueous layer was percolated through a column of Amberlite XAD-2 resin. After thorough washing with distilled water the cardiotonic glycoside fraction was eluted with methanol. The eluate was subjected repeatedly to dry column chromatography on silica gel, eluting with chloroform-methanol or methylene chloride-methanol. When further purification was necessary, high-performance liquid chromatography (HPLC) on a reversed phase column, gel chromatography on Sephadex LH-20, and preparative thin-layer chromatography (TLC) on silica gel were effective.

The first cardiotonic glycoside (Ib), mp 256—260° (dec.), $[\alpha]_D^{25}$ −4.2°, was separated as colorless prisms. Upon enzymatic hydrolysis with a *Helix pomatia* digestive juice preparation, Ib afforded hellebrigenin (Ia) as colorless prisms; this material was characterized by direct comparison with an authentic sample. The sugar moiety obtained by acid hydrolysis from Ib was identified as glucose by TLC and gas-liquid chromatography (GLC). Usual acetylation of Ib with acetic anhydride and pyridine provided the tetraacetate (Ic), mp 198—200°,

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3) M. Miyakado, T. Kato, N. Ohno, and K. Koshimizu, *Phytochemistry*, 14, 2717 (1975).
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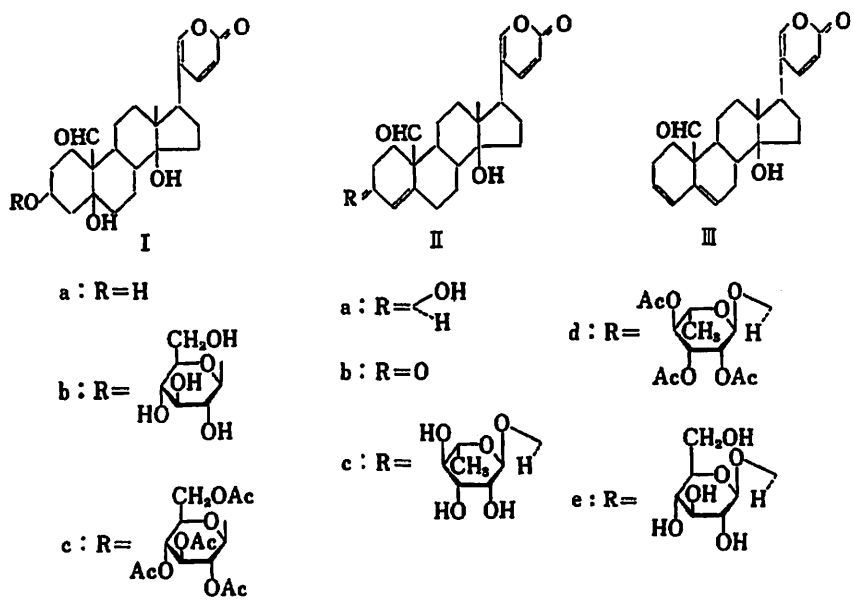

as colorless needles. Inspection of the mass spectrum revealed that Ib consists of one mole each of glucose and aglycone. The anomeric proton in the sugar moiety appeared at δ 4.65 as a doublet (J=8 Hz), indicating a *trans*-diaxial relationship to the vicinal 2′-proton. The nuclear magnetic resonance (NMR) spectrum as well as the molecular rotation[7] ($[M_{Ib}]_D - [M_{Ia}]_D = -81.9°$) were indicative of the β-glycoside linkage. These findings led us to assign the structure hellebrigenin β-D-glucoside[8] to Ib.

The second carditonic glycoside (IIc), mp 167—171° (dec.), $[\alpha]_D^{13}$ −29.4°, was obtained as a colorless amorphous substance. Hydrolysis with 1% sulfuric acid afforded anhydroscilliglaucosidin (III),[9] which was characterized by direct comparison with an authentic sample. In the NMR spectrum of IIc, the C-4 proton appeared at δ 5.85 as a diffused singlet ($W_{1/2}$=3 Hz), supporting the presence of a 3β-glycosyl-4-ene structure.[10,11] These data support the assignment of the structure scilliglaucosidin to the steroid moiety of IIc. Hydrolysis of IIc with acid yielded rhamnose, which was identified by transforming it into the *p*-nitrophenylhydrazone. The mass spectrum of the triacetate (IId), readily obtainable from IIc by usual acetylation, showed that IIc consists of one mole each of rhamnose and aglycone. The α-glycoside linkage was confirmed by inspection of the anomeric proton peak (1H, singlet) at δ 5.00 in the NMR spectrum[12] and by the difference in molecular rotation between IIc and IIa ($\Delta[M]_D$=−337°).[13,14] These findings led us to assign the structure scilliglaucosidin α-L-rhamnoside to the second cardiotonic steroid.[15,16] In similar fashion, altoside (IIe) was isolated and characterized by degradative means.

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15) Scilliglaucosidin α-L-rhamnoside was isolated from *Scilla maritima*,[16] but the details have not yet been reported.
16) Boehringer, C.H., Sohn Fr. 1539453 [*C.A.*, 71, 90050*j* (1969)].

The cardiotonic activities of steroids thus obtained were tested on isolated frog heart (Straub's preparation).[17] These compounds exhibited activity at a concentration of 10^{-6} g/ml. The cytotoxicity (ED_{50}) of these steroids against KB cell cultures was *ca.* 10^{-1}—10^{-4} μg/ml.[18]

Further studies on the isolation of cardiotonic steroids from this plant are in progress.

Experimental[19]

Extraction of Steroidal Components——Bulbs (30 kg) of *Urginea altissima* Baker collected in Kenya during Oct. 1971[20] were extracted with EtOH at room temperature for 1 month. The insoluble material was reextracted with $CHCl_3$ using a Soxhlet extractor. The $CHCl_3$ layer was concentrated *in vacuo* and the residue (500 g) was partitioned in a hexane–90% MeOH system. The MeOH layer was concentrated *in vacuo* and the residue was partitioned in a CH_2Cl_2–H_2O system. The CH_2Cl_2 layer was concentrated *in vacuo* and the residue (90 g) was chromatographed repeatedly on silica gel using AcOEt-hexane as an eluent. Purification of the eluate by preparative TLC followed by recrystallization from MeOH provided scilliglaucosidin-3-one (IIb) (100 mg), mp 275.5—281.5° (lit. mp 278—279°),[4] scilliglaucosidin (IIa) (600 mg), mp 236.6—242.1° (lit. mp 245°),[2] and hellebrigenin (Ia) (1 g), mp 236—243.7° (lit. mp 225—227°).[4] One-seventh of the ethanolic extract was partitioned in an AcOEt–H_2O system. The AcOEt layer was evaporated down *in vacuo* to give an oily residue (100 g), from which Ia (500 mg), IIa (200 mg), and IIb (50 mg) were obtained by repeated purification described above. The aqueous layer was concentrated *in vacuo* below 50° to remove the organic solvent, then diluted with H_2O (10 l). The resulting solution was percolated through a column of Amberlite XAD-2 resin (40 cm × 3.2 cm i.d.). After washing with H_2O (10 l) the desired fraction was eluted with MeOH.

Isolation of Altoside (IIe) and Hellebrigenin β-D-Glucoside (Ib)——The methanolic eluate (45 g) from Amberlite XAD-2 resin was repeatedly chromatographed on silica gel with $CHCl_3$–MeOH. IIe (500 mg) was obtained from the $CHCl_3$–MeOH (9: 1) fraction as colorless prisms. mp 226.3—228.3° (from MeOH–ether) (lit. mp 222—228°).[2] The eluate with $CHCl_3$–MeOH (9: 1.5) was purified by preparative TLC using $CHCl_3$–MeOH–H_2O (80: 20: 2.5). The adsorbent corresponding to the spot (*Rf* 0.40) was eluted with $CHCl_3$–MeOH (5: 1) and recrystallization of the eluate from acetone–MeOH gave Ib (100 mg) as colorless prisms. mp 256—260° (dec.) (lit. mp 240—244°/260—263°).[7] $[\alpha]_D^{24}$ −4.2° (c=0.14). NMR [5% solution in CD_3OD–$CDCl_3$ (1: 2)] δ: 0.70 (3H, s, 18-CH_3), 6.35 (1H, d, J=10 Hz, 23-H), 7.35 (1H, d, J=2 Hz, 21-H), 8.00 (1H, q, J=10, 2 Hz, 22-H), 10.05 (1H, s, CHO).

Acetylation of Ib——Ib (10 mg) was acetylated with Ac_2O (1 ml) and pyridine (2 ml) in the usual manner and the crude product was subjected to preparative TLC using benzene–AcOEt (1: 1) as a developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.20) with AcOEt and recrystallization of the eluate from MeOH–acetone gave the tetraacetate (Ic) (8 mg) as colorless needles. mp 198—200° (lit. mp 204—206°).[7] MS *m/e*: 746 (M^+). NMR (2.5% solution in $CDCl_3$) δ: 0.70 (3H, s, 18-CH_3), 2.00—2.20 (12H, each s, $OCOCH_3$ × 4), 3.70 (1H, m, 5′-H), 4.20 (3H, m, 3α-H, CH_2OCOCH_3), 4.65 (1H, d, J=8 Hz, 1′-H), 4.80—5.40 (3H, m, 2′,3′,4′-H), 6.30 (1H, d, J=10 Hz, 23-H), 7.30 (1H, d, J=2 Hz, 21-H), 7.80 (1H, q, J=10, 2 Hz, 22-H), 10.00 (1H, s, CHO).

Enzymatic Hydrolysis of Ib——Ib (23 mg) was incubated in MeOH (1 ml)–acetate buffer (pH 5.0, 8 ml) with acetone powder (10 mg) of *Helix pomatia* digestive juice at 37° for 2 days. The incubation mixture was then extracted with AcOEt. The organic layer was washed with 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was subjected to preparative TLC using benzene–AcOEt (1: 1) as a developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.30) with AcOEt

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18) Cytotoxicity was assayed under the auspices of the National Cancer Institute according to the procedure described by R.I. Geran, N.H. Greenberg, M.M. MacDonald, A.M. Schumacher, and B.J. Abbott [*Cancer Chemother. Rep.*, Part 3, 3, 1 (1972)].

19) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 automatic polarimeter in MeOH. Mass spectral measurements were run on a Hitachi M-52G spectrometer. NMR spectra were recorded on a JEOL PS-100 spectrometer at 100 MHz using tetramethylsilane as an internal standard. Abbreviations: s=singlet, d=doublet, q=quartet, and m=multiplet. For preparative TLC, silica gel HF_{254} (E. Merck AG, Darmstadt) and for column chromatography silica gel (70-230 mesh) (E. Merck AG) were used. HPLC was run on a Waters ALC/GPC 202/401 liquid chromatograph equipped with a μBondapak C_{18} column (Waters Assoc., Milford, Mass.). GLC was carried out on a Shimadzu GC-3BF gas chromatograph equipped with a column (1.2 m) of 1% OV-17 (Chromosorb W, 80—100 mesh) at a column temperature of 170°.

20) We thank Dr. R.E. Perdue, Jr. for supplying the plant material in accordance with a program developed by the National Cancer Institute.

and recrystallization of the eluate from MeOH gave hellebrigenin (Ia) (3 mg). Mixed melting point on admixture with an authentic sample showed no depression.

Acid Hydrolysis of Ib——A suspension of Ib (5.3 mg) in 5% H_2SO_4 (4 ml) was heated on a water-bath for 35 min. The reaction mixture was extracted with AcOEt and the aqueous layer was passed through a column of Dowex 1-X8 resin (OH^- form, 100—200 mesh). The effluent was concentrated *in vacuo*. A portion of the residue was analyzed by TLC using AcOEt–isoPrOH–H_2O (65 : 24 : 12) as a developing solvent. The remaining portion was trimethylsilylated in the usual manner and the resulting derivative was analyzed by GLC. The chromatographic behavior was identical with that of authentic glucose.

Isolation of Scilliglaucosidin α-L-Rhamnoside (IIc)——The methanolic eluate (45 g) from Amberlite XAD-2 resin was repeatedly chromatographed on silica gel with $CHCl_3$–MeOH or CH_2Cl_2–MeOH. The eluate was subjected to preparative TLC using AcOEt as a developing solvent and the adsorbent corresponding to the spot (*Rf* 0.20) was eluted with AcOEt. The eluate was further subjected to HPLC followed by gel chromatography on Sephadex LH-20 (35 cm × 1.5 cm i.d.) using MeOH as a solvent. Treatment of the eluate with MeOH–ether gave IIc (25 mg) as a colorless amorphous substance. mp 167—171° (dec.). $[\alpha]_D^{15}$ −29.4° (c=0.10). *Anal.* Calcd. for $C_{30}H_{40}O_9 \cdot 2\frac{1}{3}\,H_2O$: C, 61.42; H, 7.67. Found: C, 61.53; H, 7.50. NMR [(1.25% solution in $CDCl_3$–CD_3OD (1 : 1)] δ: 0.75 (3H, s, 18-CH_3), 1.35 (3H, d, J=7 Hz, 5′-CH_3), 5.00 (1H, s, 1′-H), 5.85 (1H, diffused s, $W_{1/2}$=3 Hz, 4-H), 6.40 (1H, d, J=10 Hz, 23-H), 7.40 (1H, d, J=2 Hz, 21-H), 8.00 (1H, q, J=10, 2 Hz, 22-H), 9.95 (1H, s, CHO).

Acetylation of IIc——IIc (10 mg) was acetylated with Ac_2O (1 ml) and pyridine (2 ml) in the usual manner and the crude product was subjected to preparative TLC using benzene–AcOEt (2 : 1) as a developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.20) with AcOEt gave the triacetate (IId) (6 mg). This compound was not obtained in crystalline form, but was essentially homogeneous as judged by TLC. MS *m/e*: 670 (M^+). NMR (1.5% solution in $CDCl_3$) δ: 0.70 (3H, s, 18-CH_3), 1.25 (3H, d, J=7 Hz, 5′-CH_3), 1.95—2.20 (9H, each s, $OCOCH_3 \times 3$), 3.80—4.20 (2H, m, 3α-H, 5′-H), 4.90 (1H, s, 1′-H), 4.95—5.40 (3H, m, 2′,3′,4′-H), 5.70 (1H, diffused s, $W_{1/2}$=3 Hz, 4-H), 6.25 (1H, d, J=10 Hz, 23-H), 7.20 (1H, d, J=2 Hz, 21-H), 7.70 (1H, q, J=10, 2 Hz, 22-H), 9.80 (1H, s, CHO).

Acid Hydrolysis of IIc——A suspension of IIc (8.3 mg) in 1% H_2SO_4 (2 ml) was heated on a water-bath for 40 min. The reaction mixture was extracted with AcOEt and the organic layer was washed with H_2O, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was subjected to preparative TLC using benzene–AcOEt (4; 1) as a developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.32) with AcOEt and recrystallization of the eluate from acetone gave anhydroscilliglaucosidin (III) (4 mg). mp 224—228° (lit. mp 220—229°).[9] NMR (1% solution in $CDCl_3$) δ: 0.70 (3H, s, 18-CH_3), 5.70—6.10 (3H, m, 3,4,6-H), 6.25 (1H, d, J=10 Hz, 23-H), 7.25 (1H, d, J=2 Hz, 21-H), 7.80 (1H, q, J=10, 2 Hz, 22-H), 9.60 (1H, s, CHO). The aqueous layer was passed through a column of Dowex 1-X8 resin (OH^- form, 100—200 mesh) and the effluent was concentrated *in vacuo*. A portion of the residue was subjected to TLC using two kinds of solvent systems (AcOEt–isoPrOH–H_2O (65 : 24 : 12), $CHCl_3$–MeOH (3 : 2)). The chromatographic behavior was identical with that of authentic rhamnose. The sugar portion was derivatized into *p*-nitrophenylhydrazone (mp 182—189°, $[\alpha]_D^{25}$ −33.3° (c=0.03)) and identified by direct comparison with an authentic sample.

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