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Lycopodium Triterpenoids. (12). Syntheses of Inundoside-A and -E, Triterpenoid-glycosides of *Lycopodium inundatum* L.[1]

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Starting from serratenediol and 21-episerratenediol, their 3-α-L-arabinopyranosides, inundoside-A and inundoside-E, triterpenoid-glycosides occurring in *Lycopodium inundatum*, were synthesized, thus proving their structures.

Keywords——Lycopodiaceae; *Lycopodium inundatum*; triterpenoid-glycoside; L-arabinoside; inundoside; serratane group; König–Knorr reaction

Previously, Tsuda *et al.*[2] described the isolation of seven triterpenoid-glycosides (inundoside-A, -B, -C, -D_1, -D_2, -E, -F, and -G) from *Lycopodium inundatum* L. (Lycopodiaceae), and reported their structure elucidation based mainly on spectral evidence. The compounds are 3-α-L-arabinopyranosides of serratenediol (A), 21-episerratenediol (E), and tohogenol (C), and their *p*-coumaroyl and/or acetyl esters (B, D_1, D_2, F, and G). Of these, we now report the syntheses of the non-acylated glycosides, inundoside-A and -E, establishing their structures.

For synthesis of inundoside-A (**1a**) (serratenediol 3-α-L-arabinopyranoside), two hydroxyl groups in serratenediol have to be differentiated so as to leave the 3β-hydroxyl free and protect the 21α-hydroxyl. Although this has been done by regioselective Grignard reaction of serratenediol diacetate (**3**) yielding serratenediol 21-monoacetate (**4**), the reaction is always accompanied by formation of the isomeric serratenediol 3-monoacetate (**5**), separation of the isomers being very inefficient.[3] Therefore, in the present synthesis, we chose serratenediol 3-monoacetate (**5**) as a starting material, since it is obtainable in pure form from *Lycopodium serratum*,[4] and decided to protect its 21α-hydroxyl group as a ketone, since hydride reduction of a triterpenoid 3-ketone (equivalent to the 21-ketone of serratane) is well known to produce an equatorial alcohol exclusively. Thus, serratenediol 3-monoacetate (**5**), was converted to serrat-14-en-3β-ol-21-one (**7**) by oxidation with pyridinium chlorochromate (PCC) followed by alkaline hydrolysis. The compound **7** was coupled with tri-*O*-acetyl-β-L-arabinopyranosyl bromide under modified König-Knorr conditions[5] to yield serrat-14-en-21-one-3β-yl α-L-arabinopyranoside (**8**). Borohydride reduction of **8** followed by purification of the product **9** through acetylation gave a tetraacetate, mp>300°C, which was identical with inundoside-A tetraacetate (**1b**). Treatment of this with methanolic NaOMe afforded inundoside-A (**1a**). The identity of this with the natural specimen was again confirmed by comparisons of their infrared (IR) spectra and thin layer chromatography (TLC) behavior.

Synthesis of inundoside-E (**2a**) was achieved as follows. Differentiation of the two hydroxyl groups of 21-episerratenediol was easier. Partial methanolysis of 21-episerratenediol diacetate (**10**) resulted in the formation of 21β-acetoxyserrat-14-en-3β-ol (**11**),[6] in which the less hindered equatorial acetoxy-group was solvolyzed and the more hindered axial acetoxy-group remained intact. Reaction of **11** with tri-*O*-acetyl-β-L-arabinopyranosyl bromide as described above yielded 21-episerratenediol 3-α-L-arabinopyranoside tetraacetate (**2b**), identical with inundoside-E tetraacetate. Treatment of this with methanolic NaOMe afforded inundoside-E. The identities of these compounds with the natural specimens were confirmed by comparisons of IR and ^{1}H-nuclear magnetic resonance (NMR) spectra, and TLC behavior.

The above syntheses provide definitive proofs of the structures of the title triterpenoid-

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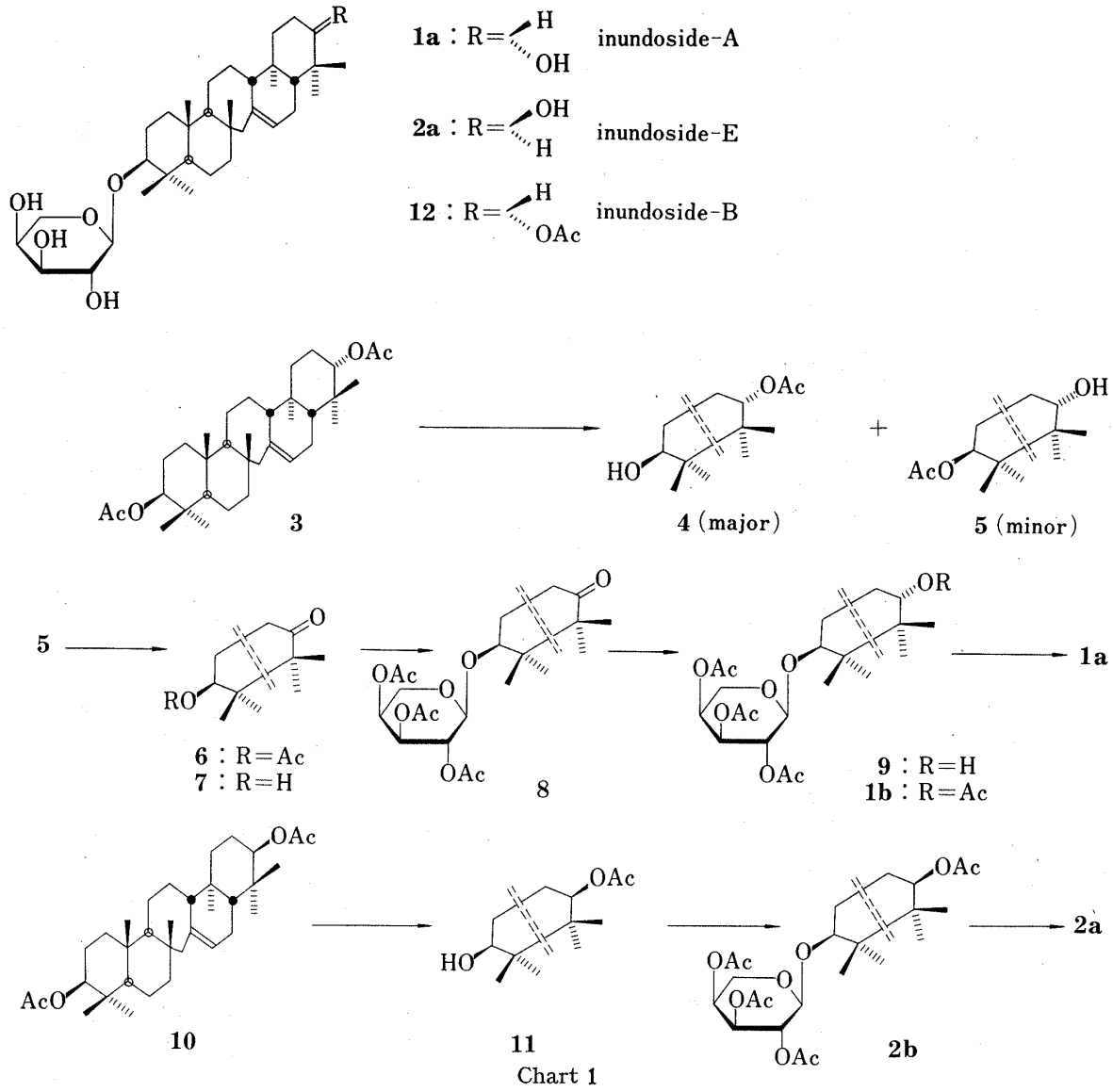

Chart 1

glycosides, and also represent a synthesis of inundoside-B (**12**), since conversion of inundoside-A tetraacetate (**1b**) to inundoside-B has already been reported.[2]

Experimental

Melting points were taken on a Yanagimoto micro hot-stage mp apparatus. The IR spectra were taken as KBr discs on a Jasco IR-G spectrometer and are given in cm^{-1}, and ^1H-NMR (100 MHz) spectra were taken in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard on a JEOL FX-100 FT NMR spectrometer.

Wakogel C-200 (silica gel) was used for column chromatography. For TLC, Kieselgel 60 F_{254} precoated plates were used and spots were developed by spraying 1% $Ce(SO_4)_2$ in 10% H_2SO_4 and heating the plates at 100°C until coloration took place.

All organic extracts were dried over anhyd. Na_2SO_4 before concentration.

Serrat-14-en-3β-ol-21-one (**7**)——Serratenediol 3-monoacetate (**5**) (100 mg) was dissolved in dry CH_2Cl_2 and stirred with PCC (150 mg) and anhyd. NaOAc (60.7 mg) at room temp. for 40 h. The mixture was applied to a column of Florisil and eluted with CH_2Cl_2 to give 3β-acetoxyserrat-14-en-21-one **6** (90 mg), mp>300°C (lit. mp 305—307°C).[4] IR: 1725, 1705. ^1H-NMR δ: $-\overset{|}{C}-CH_3$ 0.84, 0.85 (3), 0.93, 1.05, 1.09; OAc 2.04; H-3 4.39—4.55 (1H, m).

The keto-acetate **6** (115 mg) and KOH (2.5 g) in dioxane (15 ml) and MeOH (100 ml) were heated under reflux for 4 h, then the mixture was concentrated. Water was added to the residue, and the solution was

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neutralized with conc. HCl, and repeatedly extracted with $CHCl_3$–MeOH (10: 1). The combined extract was washed with water, dried, and concentrated to dryness to give **7** as colorless crystals, mp >300°C. IR: 3350 (br), 1700. This product was used for the next procedure without further purification.

Serrat-14-en-21-one-3β-yl 2′,3′,4′-Tri-*O*-acetyl-α-L-arabinopyranoside (8)——The keto-alcohol **7** (112 mg) was dissolved in dry benzene (15 ml) and nitromethane (20 ml). Benzene was distilled off to remove moisture azeotropically. $Hg(CN)_2$ (126 mg) was added to the cooled mixture, followed by the addition of tri-*O*-acetyl-β-L-arabinopyranosyl bromide (170 mg) and anhyd. $CaSO_4$ (112 mg), and the mixture was heated at 115°C for 6 h. After cooling, the mixture was filtered and the residue was washed with $CHCl_3$. The combined filtrate and washings were diluted with $CHCl_3$, and shaken well with sat. $NaHCO_3$ solution, then the $CHCl_3$ layer was washed with water, dried, and concentrated to leave a solid. This was subjected to chromatography and eluted successively with benzene and CH_2Cl_2. The benzene eluate gave the starting material **7** (43.5 mg) and the CH_2Cl_2 eluate gave **8** (64.5 mg), mp >300°C, as colorless needles from CH_2Cl_2–MeOH. IR: 1724, 1705, 1235. ^{1}H-NMR δ: –C–CH_3 0.75, 0.80, 0.82, 0.92 (2), 1.04, 1.08; OAc 2.01, 2.04, 2.13; H-3 3.0 (1H, m), H-5′ 3.59 (1H, dd, J=13.0 and 1.5 Hz); H-5′ 4.01 (1H, dd, J=13.0 and 2.4 Hz); H-1′ 4.44 (1H, d, J=7 Hz); H-2′ H-3′ H-4′ 4.95—5.38 (3H); –CH= 5.38 (1H, m). *Anal.* Calcd for $C_{41}H_{62}O_9 \cdot 1/2H_2O$: C, 69.55; H, 8.97. Found: C, 69.78; H, 8.83.

21α-Hydroxyserrat-14-en-3β-yl 2′,3′,4′-Tri-*O*-acetyl-α-L-arabinopyranoside (9) (Inundoside-A Triacetate)——The above compound **8** (64 mg) and $NaBH_4$ (10 mg) in tetrahydrofuran (2 ml) and MeOH (8 ml) were stirred at 0—5°C for 20 min then at room temp. for a further 20 min. The cooled mixture was slightly acidified with conc. HCl and concentrated *in vacuo* to dryness. Water was added to the residue and the solution was extracted with $CHCl_3$. The extract was washed with water, dried, and concentrated to give **9** (60.5 mg), which crystallized from CH_2Cl_2–MeOH in fine needles, mp >300°C. IR: 3430, 1725. ^{1}H-NMR (60 MHz) δ: –C–CH_3 0.66, 0.78, 0.82, 0.83 (2), 0.91, 0.96; OAc 2.02, 2.04, 2.10; H_2-5′ 3.50—3.85 (2H); H-1′ 4.50 (1H, d, J=7 Hz); –CH= 5.25 (1H, bs). *Anal.* Calcd for $C_{41}H_{64}O_9 \cdot 1/2H_2O$: C, 69.43; H, 9.23. Found: C, 69.10; H, 9.12.

Inundoside-A (1a)——The above triacetate **9** (60 mg) in Ac_2O (1 ml) and pyridine (2 ml) was kept overnight at room temp. Water was added to the mixture and the solution was extracted with CH_2Cl_2. Concentration of the dried extract gave a residue, which was dissolved in CH_2Cl_2 and passed through a short silica gel column to give the tetraacetate **1b** (40 mg), mp >300°C, as colorless needles from $CHCl_3$–MeOH. *Anal.* Calcd for $C_{43}H_{66}O_{10}$: C, 69.54; H, 8.90. Found: C, 69.59; H, 9.12.

The identity of this product with natural inundoside-A tetraacetate (**1b**) was confirmed by comparisons of ^{1}H-NMR spectra and TLC behavior.

Treatment of the above acetate **1b** (10 mg) with 0.2 N NaOMe (2 ml) in dry MeOH (10 ml) at 50°C for 2 h, and work-up as described previously[2] gave inundoside-A (**1a**) (5 mg), mp >300°C, colorless needles from $CHCl_3$–MeOH, as confirmed by comparisons of IR spectra and TLC behavior.

21β-Acetoxyserrat-14-en-3β-ol (11)——21-Episerratenediol diacetate (**10**) (100 mg) and 0.2 N NaOMe (4 ml) in dry $CHCl_3$ (2 ml)–MeOH (2 ml) were stirred at room temp. for 26 h. The mixture was slightly acidified with AcOH and concentrated to dryness. Water was added to the residue and the solution was extracted with $CHCl_3$. The extract was washed with water, dried, and concentrated to give a gummy residue which was chromatographed and eluted with benzene, $CHCl_3$, $CHCl_3$–MeOH (10: 1). The benzene eluate gave the starting material **10** (70 mg). The $CHCl_3$ and $CHCl_3$–MeOH eluates gave the 21-monoacetate (**11**) (30 mg), mp 224—226°C (lit. mp 240—244°C).[6] The identity of this product with the authentic specimen was confirmed by comparison of the ^{1}H-NMR spectra.

Repetition of the above procedure with the recovered starting material gave a further crop (27 mg) of the monoacetate **11**.

Inundoside-E (2a)——The monoacetate **11** (56 mg) was dissolved in dry benzene (10 ml) and dry nitromethane (15 ml). Benzene was distilled off to remove moisture azeotropically, then the mixture was treated with $Hg(CN)_2$ (91 mg), tri-*O*-acetyl-β-L-arabinopyranosyl bromide (122 mg), and dry $CaSO_4$ (60 mg) at 115—117°C for 7 h, and worked up in the manner described for **8**. The crude product was subjected to chromatography and eluted with benzene, CH_2Cl_2, and $CHCl_3$–MeOH (10: 1). The CH_2Cl_2 and CH_2Cl_2–MeOH eluates gave the tetraacetate **2b** (52 mg), mp 293—293.5°C (lit. mp 291—293°C),[2] as colorless needles from CH_2Cl_2–MeOH. The identity of this product with inundoside-E tetraacetate was confirmed by comparisons of ^{1}H-NMR spectra and TLC behavior.

The above tetraacetate **2b** (10 mg) and 0.2 N NaOMe (2 ml) in MeOH (10 ml) was heated at 60°C for 2 h. The mixture was neutralized with Amberlite IRA-120-H^+, filtered, and the resin was washed with $CHCl_3$–MeOH. Concentration of the combined filtrate and washings to dryness left a solid which, on crystallization from $CHCl_3$–MeOH, gave inundoside-E (**2a**), mp >300°C, as colorless needles.

The identity of this product with the natural specimen was confirmed by comparisons of IR spectra and TLC behavior.

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