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Sterols and Triterpenoids from *Codonopsis lanceolata*

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Abstract—The roots of *Codonopsis lanceolata* contained α-spinasterol, Δ^7-stigmastenol, oleanolic acid, echinocystic acid, and an unidentified triterpene acid, mp 249°.

Codonopsis lanceolata BENTH et HOOKER (Campanulaceae) is a perennial herb, roots of which are known as both edible and medicinal plant, being used as an expectorant. Although anti-inflammatory activity of this plant was reported[1], these active principles have not been known yet. In this paper, isolation and idenification of sterols and triterpenes in this plant are described.

Sterols—Phytosterol mixture, mp 136°, was isolated from unsaponifiable fraction of the ether soluble extract. It showed positive Lieberman-Burchard reaction (very rapid color change from pink to blue green). Its IR absorption spectrum shows OH band at 3400cm^{-1} and absorption bands due to double bond at 1650, 970 (*trans*-disubstituted double bond) and 825~850cm^{-1} (trisubstituted double bond). Although this substance showed single spot on silica gel plates with or without $AgNO_3$ and the spot was superimposible on that of the authentic sample of α-spinasterol, its mass spectrum exhibits another parent peak at m/e 414 besides peaks which are characteristic of α-spinasterol[2]. GLC analysis conformed that this substance was a mixture of α-spinasterol and Δ^7-stigmastenol (27 : 73).

Triterpenoids—Three substances(compound A~C) were obtained from the hydrolysate of the crude saponin.

Compound A, mp 310°, was identified to be oleanolic acid by direct comparison with authentic sample (mixed mp, TLC, IR, and MS), and preparation of methyloleanolate(methylation with CH_2N_2) and 3-acetyloleanolic acid (acetylation with Ac_2O and pyridine).

Compound B(I), $C_{30}H_{48}O_4 \cdot H_2O$, mp 303~308°, showed positive Lieberman-Burchard reaction and tetranitromethane test. Its IR specctrum shows OH band at 3560cm^{-1}, carboxyl

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band at 1700 cm^{-1}, and trisubstituted double bond at 810~830 cm^{-1}. Acetylation of **I** with acetic anhydride-pyridine gave a diacetate(**II**), mp 271~275°. **I** was esterified on treatment with ethereal diazomethane to give a methyl ester(**III**), mp 206~208°, which was hydrolysed by 4% KOH to give **I**(30%).

Methylation of **II** or acetylation of **III** gave equally a methyl diacetate(**IV**), mp 197~199°, which consumed one mole of perbenzoic acid. Oxidation of **IV** with CrO_3-HOAc afforded an α,β-unsaturated ketone(**V**), mp 220~223° and treatment of **III** with Br_2-HOAc gave a bromo-γ-lactone(**VI**), mp 141~145°. On the basis of the above experiments, this compound is supposed to be a dihydroxy-olean-12-en-28-oic acid.

Mass spectra of **I**, **II**, and **III** show typical retro-Diels-Alder fragmentation and the rest of the peaks due to further cracking are similar to the usual cascading patterns expected for olean-12-ene series[3]. Mass spectra analysis together with the saponification rate* of carbomethoxyl group of **III** suggest that one of hydroxyl groups should be located either at C-16 or C-22.

The IR spectrum of **III** exhibits strong bands at 1730 cm^{-1}, showing no intramolecular hydrogen bonding between hydroxyl and carbonyl groups[4,5], thus suggesting the presence of an axial hydroxyl group at C-16 in **I**. The presence of a second hydroxyl group at C-3 is more probable from biogenetic ground. Its equatorial(β) orientation is supported by the presence of a triplet-like multiplet(1H) centered at δ 4.50 in the NMR spectrum of **IV** with splitting pattern characteristic of the proton α to the 3β-acetoxyl group[6~9]. Observed values in PPM(0.72, 0.86×2, 0.94, 0.98×2, and 1.25) of the chemical shifts for the C-methyl groups of **IV** are in excellent agreement with the values (0.69, 0.87×2, 0.95, 0.98, 0.99, and 1.26 for Me 26, 23, 24, 25, 29, 30, and 27, respectively) calculated for 3β, 16α-diacetoxyolean-12-en-28-oic acid methyl ester based on the data presented by Tursch, *et al.*[10] and Ito, *et al.*[11] The value of the width at half-height of signal at 5.63 is *ca* 6 Hz, being in agreement with the assigned stereochemistry of an acetoxyl group as 16α.

Although the direct comparison with authentic sample has been unavailable, the accumulated evidences for compound B clearly assure its identity with echinocystic acid.

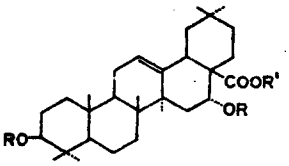
Compound C, mp 249~251°, (M^+, *m/e* 472), was assumed to be an isomer of compound B, however, further examination was not undertaken due to shortage of the material.

EXPERIMENTAL*

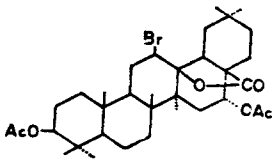
Extraction and Hydrolysis of Crude Saponin—MeOH extracts of the dried roots

* It is known that the rate of saponification of the triterpene esters of the oleanane series having the carbomethoxyl at the C-17 position is very slow but the presence of the hydroxyl group β to the carbomethoxy group facilitates the hydrolysis[12].

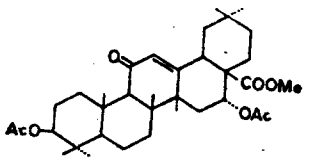
** The melting points were determined in open capilaries in sulfuric acid and are uncorrected. Optical rotations were measured in MeOH. IR spectra were recorded in KBr and UV spectra in EtOH. NMR spectra were taken in $CDCl_3$ using TMS as internal reference at 100 MHz. We are grateful to Dr. D.Y. Han, College of Pharmacy, Jung Ang University, for the measurements of the mass spectra.



I R = R' = H
II R = Ac, R' = H
III R = H, R' = Me
IV R = Ac, R' = Me



V



VI

(10 kg) were partitioned between Et_2O and water, and water layer was extracted with BuOH. After removal of solvent, the crude saponin was precipitated by addition of Et_2O into MeOH soln. of the residue(yield 15g). The crude saponin(3g) was hydrolyzed by refluxing in 5% MeOH-H_2SO_4 for 5hr. The aglycone fraction(1g) precipitated by addition of water was chromatographed on silica gel and elution with MeOH-$CHCl_3$ (1 : 100 to 1 : 20) gave 3 components, which have been designated as compound A, B, and C in order of increasing polarity on TLC ($CHCl_3$-MeOH-7% HOAc=5 : 1 : 1).

Compound A, mp 310°, IR 3400 cm^{-1}(OH), 1680 cm^{-1}(COOH), and 825 cm^{-1} (trisubstituted double bond). MS *m/e* 456(M^+ 1.7), 248(100), 207(40.7), 203(98), 189(25.9), and 133(37), was identified as oleanolic acid by direct comparison with the authentic specimen.

Methylation with CH_2N_2 and acetylation with Ac_2O and pyridine of compound A gave a methylester, mp 198~200° and an acetate, mp 290~300°, which did not depress the mps of authentic samples of methyl oleanolate and acetyl oleanolic acid, respectively.

Compound B(I), mp 303~308°, $[\alpha]_D$=+15.7° (c, 0.22 in MeOH), λ_{max}^{EtOH} 204 nm (log ε, 3.80), IR 3560 cm^{-1}(OH), 1700 cm^{-1}(COOH), and 810~830 cm^{-1} (trisubstituted double bond); NMR δ 0.77(s, 3H), 0.79(s, 3H), 0.88(s, 3H), 0.97(s, 9H), 1.38(s, 3H), 3.27~3.33 (m, 1H), and 5.5(m, 1H). MS *m/e* 472(M^+ 25), 264(52), 246(100), 219(17), 207(52), 201(67), 189(48), 149(9.8), and 131(25).

Anal. Calcd for $C_{30}H_{48}O_4 \cdot H_2O$: C, 73.47; H, 10.20. Found: C, 73.98; H, 10.28.

Compound C, mp 249~251°, IR 3540 cm^{-1} (OH) and 1698 cm^{-1} (COOH), MS *m/e* 472 (M^+).

Acetylation of I—A sample of I (30 mg) was acetylated by boiling with Ac_2O (2 ml) and pyridine (4 ml). The mixture was worked up in the usual manner and after recrystallization from MeOH yielded II, mp 271~275°, IR 1740 and 1250 cm^{-1} (acetate); NMR δ 0.73(s, 3H), 0.85(s, 6H), 0.93(s, 6H), 0.99(s, 3H), 1.27(s, 3H), 2.05(s, 3H), 2.09(s, 3H),

4.5(t like, 1H), 5.69(t like, 1H), and 5.42(t like, 1H). MS *m/e* 556(M^+ t), 249(13), 246(100), 201(33), 191(20), 189(67), and 131(46).

Methyl Ester(III)—A sample of **I** (600 mg) was treated with diazomethane in the usual manner. After working up in the usual way, **III** was crystallized from MeOH as fine needles, mp 206~208°, $[\alpha]_D^{26}$=+26.6° (c, 0.52 in MeOH), IR 1730 cm^{-1}(ester), MS *m/e* 486 (M^+ 25), 278(48), 260(100), 149(20), 207(13), 201(33), 189(67), and 131(46). **I** was recovered in 30% yield by saponification of **III** with 4% MeOH-KOH for 5 hr.

Methyl Diacetate(IV)—Methylation of **II**, or acetylation of **III** under similar condition as above gave **IV**, which was crystallized from MeOH to give fine needles, mp 197~199°, IR 1750 cm^{-1} (ester) and 1240cm^{-1} (acetate), NMR δ 0.72(s, 3H), 0.86(s, 6H), 0.94 (s, 3H), 0.98(s, 6H), 1.25(s, 3H), 2.04(s, 3H), 2.08(s, 3H), 3.62(s, 3H), 4.50(t like 1H), 5.65(t like 1H), and 5.40(t like 1H).

CrO_3-Oxidation of the Methyl Diacetate(IV)—To a boiling solution of **IV**(70 mg) in HOAc(20ml), CrO_3(100 mg) in 85% HOAc(10ml) was added dropwise over a period of 1 hr. Refluxing was continued further for an additional 90 min and then the mixture was poured onto ice. The solids were filtered and chromatographed on silica gel. Elution with $CHCl_3$ gave **V** as needles, mp 220~223°, λ_{max}^{EtOH} 250 nm (log ε, 4.00).

Bromolactone(VI) of II—To a solution of **II**(35 mg) and NaOAc(300 mg) in HOAc (10 ml) was added dropwise a solution of bromine in HOAc(3%, 2 ml). It was kept at room temperature for 2hr and mixture then poured into water(50 ml) containing $Na_2S_2O_3$ (1 g) to discharge excess bromine. The precipitate was filtered, washed thoroughly with water and dried. Crystallization from MeOH gave needles, mp 141~145°, IR 1760 cm^{-1} (γ-lactone).

Isolation of Sterol Mixture—The Et_2O soluble fraction was saponified with 5% MeOH-KOH for 2hr. After filtering filtrate was diluted with water and extracted with Et_2O. The repeated recrystallization of the unsaponifiable matter from MeOH gave phytosterol as colorless plates, mp 136°, IR 3450 cm^{-1} (OH), 970 cm^{-1} (*trans*-disubstituted double bond), and 825 cm^{-1} (trisubstituted double bond), MS *m/e* 414 (M^+ 41.5 for Δ^7-stigmastenol), 412 (M^+ 48.1 for α-spinasterol), 399(15), 397(21.6), 369(26.6), 351(23.2), 300(25), 273(56.4), 271 (100), 255(93.8), 246(44.8), and 231(39); NMR δ 0.54(s, 3H), 0.79(s, 3H), 1.02(d, 3H), 3.50(b, 1H), and 5.0~5.3(m, 2H).

Gas-Liquid Chromatography of Sterol Mixture—The chromatographic column was 100 cm×3mm glass and contained 3% OV-17 on Chromosorb W(60~80 mesh). Column temp., 260°; injection temp. and detector temp., 275°; carrier gas, N_2(40 ml/min). Cholesterol was run with the samples. RRT, Cholesterol, 1; α-spinasterol, 1.72; Δ^7-stigmastenol, 1.95.

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