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Studies on the Constituents of Zizyphi Fructus. I. Structure of Three New *p*-Coumaroylates of Alphitolic Acid

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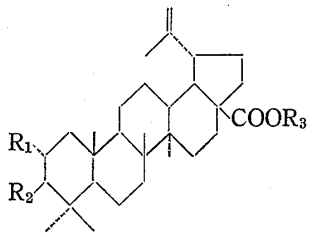
Three new *p*-coumaroylates of alphitolic acid were isolated from the fruits of *Zizyphus jujuba* (Rhamnaceae) and were characterized to be 3-O-*trans*-*p*-coumaroyl-, 2-O-*trans*-*p*-coumaroyl- and 3-O-*cis*-*p*-coumaroyl alphitolic acid on the basis of chemical and spectral evidence.

Keywords——*Zyziphus jujuba*; Rhamnaceae; three new *p*-coumaroylates of alphitolic acid; 3-O-*trans*-*p*-coumaroyl alphitolic acid; 2-O-*trans*-*p*-coumaroyl alphitolic acid; 3-O-*cis*-*p*-coumaroyl alphitolic acid

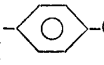
Several plants of *Zizyphus* genus(Rhamnaceae) have been used as a crude drug for the cure of biliousness, chronic bronchitis, consumption and blood diseases or as analeptic or expectorant. The above ground parts or root barks of *Zizyphus* species have been reported to contain triterpenoid acids; betulinic acid, oleanolic acid, alphitolic acid, 2α-hydroxyursolic acid, ceanothic acid[2] or peptide alkaloids; mauritine A, mucronine D, amphibine H, nummularine A and B, jubanine A and B.[3] As for the ingredients of the fruits Tomoda, *et al.* isolated D-glucose, D-fructose, oligosaccharide and polysaccharide from the water-soluble fraction of *Zizyphus jujuba*.[4] On the study of pharmacological active principles in the fruits of *Zizyphus jujuba* Mill. we isolated three new *p*-coumaroylates of alphitolic acid. This paper concerns the structure elucidation of these esters along with known triterpenoid acids.



I : R_1=H, R_2=OH, R_3=H
Ia: R_1=H, R_2=OH, R_3=Me
II : R_1=R_2=OH, R_3=H
IIa: R_1=R_2=OAc, R_3=H
IIb: R_1=R_2=OH, R_3=Me
IIc: R_1=R_2=OAc, R_3=Me

III : R_1=OH, R_2=O-CO-CH=CH-  -OH, R_3=H

IIIa: R_1=OH, R_2=O-CO-CH=CH-  -OMe, R_3=Me

IV: R_1=O-CO-CH=CH-  -OH, R_2=OH, R_3=H

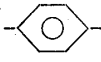
V : R_1=OH, R_2=O-CO-CH=CH-  -OH, R_3=H

Fig. 1

1) Location: *a) Maidashi, Higashi-ku, Fukuoka; b) Tashiro, Tosu, Saga.*
2) M. Ikram and H. Tomlinson, *Planta Med.*, **29**, 289 (1976).
3) R. Tschesche, I. Khokhar, H. Wilhelm, and G. Eckhardt, *Phytochemistry*, **15**, 541 (1976).
4) M. Tomoda, M. Takahashi, and S. Nakatsuka, *Chem. Pharm. Bull.* (Tokyo), **21**, 707 (1973).

The dried fruits were treated as described in the experimental section to yield compound A–E.

Compound A (I), mp 293—295° (sublimate at 265°), $[\alpha]_D^{23}$ +6.6° (pyridine), *m/e* 456 (M^+) showed blue-violet for H_2SO_4 reagent and indicated carboxylic acid and olefinic absorption bands on the infrared (IR) spectrum. On the nuclear magnetic resonance (NMR) spectrum I indicated the signals due to five tertiary methyl, isopropenyl and methine protons in triterpenoid acid skelton. On treatment with diazomethane I afforded monomethyl ester, Ia, mp 217—218°. The above physical and spectral data suggested I to be betulinic acid. By comparison of the NMR and IR spectra of Ia to those of an authentic sample I was identified to be betulinic acid.[5]

Compound B (II), mp 275—278°, $[\alpha]_D^{19}$ −4.0° (pyridine), $C_{30}H_{48}O_4$, *m/e* 472 (M^+), showed characteristic blue for H_2SO_4 reagent and indicated carboxylic acid and olefinic absorption bands on the IR spectrum. The NMR spectrum of II exhibited two methine proton signals due to C_3–H and C_2–H at δ 3.03 and 3.72, respectively together with the signals corresponding to five tertiary methyl and isopropenyl groups. On acetylation with Ac_2O and pyridine II afforded diacetate IIa, mp 240—243°, $C_{34}H_{54}O_6$. The NMR spectrum of IIa exhibited two acetyl methyl and two methine proton (C_3–H and C_2–H) signals at δ 1.96, 2.04 and 4.69, 5.06, respectively along with the signals due to five methyl and isopropenyl groups in II. On treatment with diazomethane II afforded monomethyl ester IIb, mp 247—249°, $C_{31}H_{50}O_4$, showing ester absorption band (1725 cm^{-1}) on the IR spectrum. The NMR spectrum of IIb indicated the proton signal due to a methoxycarbonyl group at δ 3.64 besides the signals corresponding to five tertiary methyl and isopropenyl groups in II. The methyl ester diacetate (IIc) of II, mp 230—234°, $C_{35}H_{54}O_6$, showed ester absorption band (1730 cm^{-1}) on the IR spectrum and the proton signals due to a methoxycarbonyl (δ 3.65), two methine protons at C_3 and C_2 (δ 4.70 and 5.20, respectively) and two acetyl methyls (δ 1.98 and 2.06) on the NMR spectrum. The examination on the mass fragment ion peaks of II and its derivatives suggested II to be alphitolic acid. Furthermore, the diequatorial 2α, 3β-configuration of hydroxyl groups in II is confirmed by examination of the coupling constant ($J_{2a,1a}=J_{2a,3a}$ =10 Hz; $J_{2a,1e}$=4 Hz) on the NMR spectrum of II and its derivatives. The above physical and spectral data of II and its derivatives are fairly consistent with those of authentic alphitolic acid and its derivatives.[6,7] Thus, the structure of II was determined to be alphitolic acid.

Table I. Nuclear Magnetic Resonance Spectral Data of C_2 and C_3 Methine Protons in II, IIa, b, c, III, IV and V

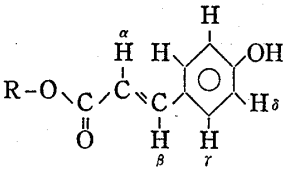
	II	IIa	IIb	IIc	III	IV	V	I	Ia
C_2	3.72[a]	5.06[b]	3.63[b]	5.20[b]	3.96[a]	5.16[a]	3.88[a]		
	a	a	d	a	a	a	a		
C_3	3.03	4.69	2.96	4.70	4.77	3.33	4.66	3.25[a]	3.08[b]
	b	b	b	b	b	b	b	c	c

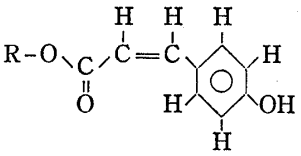
a, sextet, J=10;10;4 Hz b, doublet, J=10 Hz c, multiplet,[c] d, overlapped.
The spectra were determined in $CDCl_3$– pyridine-d_5(10: 1)(*a*) or $CDCl_3$(*b*) with Me_4Si as an internal standard at 100 MHz.
c) The signal is overlapped by the signal of C_{19}–H.

Compound C (III), mp 279—282°, $[\alpha]_D^{28}$ −33° (pyridine), $C_{39}H_{54}O_6$, *m/e* 618 (M^+), showed characteristic blue for H_2SO_4 and orange for benzidine reagents. III showed α,β-unsaturated carbonyl ester (1690 cm^{-1}) and carboxylic acid (1705 cm^{-1}) absorption bands on the IR spectrum and the absorption band due to phenolic moiety (312 nm) on the ultraviolet (UV) spectrum. On the NMR spectrum III exhibited the signals due to the AB type signal of

5) S.R. Bhattacharjee and A. Chatterjee, *J. Indian Chem. Soc.*, **39**, 276 (1962).
6) H.T. Cheung and D.G. Williamson, *Tetrahedron*, **25**, 119 (1969).
7) H.T. Cheung and M.C. Feng, *J. Chem. Soc.* (*C*), **1968**, 1047.

Table II. Nuclear Magnetic Resonance Spectral Data of *p*-Coumaroyl Protons in III, IV and V





	α	β	γ	δ
III	6.28(J=16)	7.63(J=16)	7.33(J=8)	6.89(J=8)
IV	6.19(J=16)	7.56(J=16)	7.29(J=8)	6.89(J=8)
V	5.83(J=12)	6.82(J=12)	7.72(J=8)	6.88(J=8)

R=alphitolic acid.
The spectra were determined in $CDCl_3$–pyridine-d_5(10: 1) with Me_4Si as an internal standard at 100 MHz.

aromatic protons at δ 6.89 (J=8 Hz) and 7.33 (J=8 Hz) and *trans* olefinic protons conjugated with aromatic ring (δ 6.28 and 7.63, J=16 Hz) of which a proton at β-position to carbonyl group appeared in the lower-field together with the signals due to the proton of II. On the basis of these finding III is assumed to be *p*-coumaroyl ester of II. On alkaline hydrolysis III gave II and *p*-coumaric acid which were identified with authentic samples. On the mass spectrum of III the fragment ion formed by a loss of 46 mass units (COOH, H) from the molecular ion appeared at *m/e* 572.[2] On treatment with diazomethane III afforded methyl ester IIIa along with methyl ester of contaminated V. On the NMR spectrum two methoxycarbonyl and methoxy proton signals appeared at δ 3.63 (or 3.78) and 3.92 (or 3.98). The above results provide that III is C_2 or C_3 *p*-coumaroyl ester of II. The linkage of *p*-coumaric acid in III was determined to be C_3 position by the comparison of chemical shifts of C_2- and C_3–H in II and its derivatives on the NMR spectrum. Therefore, the structure of III was established to be 3-O-*trans*-*p*-coumaroyl alphitolic acid.

Compound D(IV), mp 279—280°, $[\alpha]_D^{28}$ −1.7° (pyridine), $C_{39}H_{54}O_6$, *m/e* 618 (M^+), gave same positive H_2SO_4 and benzidine tests to those of III. IV indicated α,β-unsaturated carbonyl ester (1690 cm^{-1}) and carboxylic acid (1705 cm^{-1}) absorption bands on the IR spectrum and the presence of phenolic moiety (308 nm) on the UV spectrum. The NMR spectrum of IV revealed the AB type aromatic proton signals (δ 6.89 and 7.29, J=8 Hz), *trans* conjugated olefinic proton signals (δ 6.19 and 7.56, J=16 Hz) and the signals due to C_3- and C_2–H (δ 3.33 and 5.16, respectively), together with the signals due to the protons of II. On alkaline hydrolysis IV afforded *p*-coumaric acid and II which were identified with authentic samples. On the mass spectrum of IV the mass fragment ion peak (*m/e* 572) can be explained in terms of a loss of 46 mass units (COOH, H) from the molecular ion. Thus, IV was demonstrated to be C_2 or C_3 *p*-coumaroyl ester of II. The linkage of *p*-coumaric acid in IV was determined to be C_2 position by the comparison of the chemical shifts of C_2- and C_3–H in IV with those of II and III on the NMR spectrum. Accordingly, the structure of IV was established to be 2-O-*trans*-*p*- coumaroyl alphitolic acid.

Compound E (V), mp 208—210°, $[\alpha]_D^{24}$ +40° (pyridine), $C_{39}H_{54}O_6$, *m/e* 618 (M^+), gave same positive H_2SO_4 and benzidine tests to those of III and IV. V showed α,β-unsaturated carbonyl ester (1685 cm^{-1}) and carboxylic acid (1715 cm^{-1}) absorption bands on the IR spectrum and the presence of phenolic moiety (310 nm) on the UV spectrum. The NMR spectrum of V exhibited *cis* conjugated olefinic proton signals (δ 5.83 and 6.82, J=12 Hz) instead of those of *trans* in III and the proton at C_2, and C_6, in aromatic ring appeared in the lower-field (δ 7.72), along with the signals due to the protons of II. On alkaline hydrolysis V afforded *p*-coumaric acid and II on TLC. Thus, the structure of V was verified to be *cis* *p*-coumaric acid ester of II. The NMR and mass spectral data of V was compared with those

of III and IV to find that the linkage of ester is to be C_3 position. In addition, on heating at 100° for 24 hr V was converted to III which was identified by direct comparison (NMR, IR and TLC). These results led to the conclusion that *cis p*-coumaroyl moiety is located at C_3 position in II. Consequently, the structure of V was established to be 3-O-*cis*-*p*-coumaroyl alphitolic acid. III, IV and V are the first *p*-coumaroylates of alphitolic acid ever reported and the presence of *cis*-*p*-coumaroyl ester in nature is of significance.

Experimental

Melting points were determined on a Yanagimoto melting point apparatus and uncorrected. IR spectra were obtained with a KOKEN DS-301 and UV spectra were recorded with a Hitachi ESP-3T automatic recording spectrophotometer. NMR spectra were taken with a JEOL C-100H spectrometer and chemical shifts are given in δ scale with Me_4Si as an internal standard and coupling constants (J) in Hz. Abbreviation used, s, singlet; d, doublet; t, triplet; q, quartet; sex, sextet; m, multiplet; br, broad. Unless otherwise indicated solvent used was $CDCl_3$–pyridine-d_5 (10: 1). Mass spectra were recorded on a JMS-01SG mass spectrometer with an accelerating potential of 6.3 kV, an ionizing potencial of 75 eV and a source temperature of 190°. Thin-layer chromatography (TLC) was performed on Kieselgel G (Merck) using solvent system, C_6H_6–acetone (10: 1) for I and derivatives of II, C_6H_6–acetone (5: 1) for III—V and C_6H_6–acetone (3: 1) for II. Column chromatography was performed with Kieselgel 60 (70—230 mesh) (Merck).

Isolation of Triterpenoids——The dried fruits (20 kg) of *Zizyphus jujuba* were extracted with boiling EtOH for 1 hr, twice. The EtOH extract (11.2 kg) suspended in H_2O was extracted with BuOH. The BuOH extract (240 g) was chromatographed over silica gel using C_6H_6–acetone (7: 3) as solvent to give resinous substance (112 g). The resinous substance was partitioned between 2% Na_2CO_3 and EtOAc and the EtOAc layer was evaporated to dryness. The residue (64 g) was chromatographed over silica gel using $CHCl_3$–EtOAc as solvent to give fraction A (25.8 g) [eluation with $CHCl_3$–EtOAc (95: 5)], fraction B (9.6 g) [eluation with $CHCl_3$–EtOAc (85: 5)] and fraction C (19.2 g) [eluation with $CHCl_3$–EtOAc (1: 1)].

Isolation of Betulinic Acid (I)——Fraction A (25.8 g) was subjected to the repeated chromatographies using $CHCl_3$–EtOAc as solvent and the fraction eluted with $CHCl_3$–EtOAc (10: 1) was recrystallized from $CHCl_3$–MeOH to give colorless needles I (968 mg), mp 293—295°, $[\alpha]_D^{23}$ +6.6° (pyridine, c=2.5), IR ν_{max}^{KBr} cm^{-1}: 3440, 1690, 1640, 1050, 890. NMR δ: 0.83, 0.99, 1.01, 1.04 (15H, each s, CH_3), 1.73 (3H, s, C_{30}–CH_3), 3.10, 3.25 (2H, m, C_3–, C_{19}–H), 4.63 (1H, d, J=2, C_{29}–H), 4.79 (1H, d, J=2, C_{29}–H), MS m/e: 456 (M^+), 438 (M^+-H_2O), 423, 410 (M^+—COOH, —H).

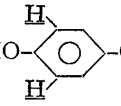
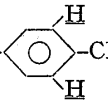
Betulinic Acid Methyl Ester (Ia)——I (70 mg) dissolved in MeOH–$CHCl_3$ (1: 1) was methylated with CH_2N_2 at room temperature to give colorless needles Ia (40 mg) (recrystallized from $CHCl_3$–MeOH), mp 217—218°. NMR ($CDCl_3$) δ: 0.75, 0.82, 0.92, 0.96 (15H, each s, CH_3), 1.69 (3H, s, C_{30}–CH_3), 2.98 ,3.08 (2H, m, C_3-H, C_{19}–H), 4.58 (1H, d, J=2, C_{29}–H), 4.71 (1H, d, J=2, C_{29}–H). The IR and NMR spectra of Ia were superimposed with those of an authentic sample.

Isolation of Alphitolic Acid (II)——Fraction C (19.2 g) was subjected to repeated chromatographies using C_6H_6–acetone as solvent and the fraction eluted with C_6H_6–acetone (3: 1) was recrystallized from $CHCl_3$–MeOH to give colorless needles II (1.15 g), mp 275—278° (dec.). $[\alpha]_D^{19}$ −4.0° (pyridine, c=1.0), MS m/e: 472.3598 (M^+, Calcd. for $C_{30}H_{48}O_4$ 472.3553), 454 (M^+-H_2O), 426 (M^+—COOH, —H). IR ν_{max}^{KBr} cm^{-1}: 3400, 1690, 1640, 1050, 890. NMR δ: 0.84, 0.88, 0.97, 1.00, 1.04 (each s, CH_3), 1.74 (3H, s, C_{30}–CH_3), 3.03 (1H, d, J=10, C_3–H), 3.72 (1H, sex, J=10; 10; 4, C_2–H), 4.60 (1H, d, J=2, C_{29}–H), 4.76 (1H, d, J=2, C_{29}–H).

Diacetyl Alphitolic Acid (IIa)——II (30 mg) was acetylated as usual way to give colorless amorphous powders of diacetate IIa (22.6 mg), mp 240—243° (recrystallized from EtOH), MS m/e: 556.3797 (M^+, Calcd. for $C_{34}H_{54}O_6$ 556.3764), 538 (M^+-H_2O), 510 (M^+—COOH, —H). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735, 1695, 1640, 1260, 1040, 890. NMR ($CDCl_3$) δ: 0.86, 0.92, 0.96 (15H, each s, CH_3), 1.68 (3H, s, C_{30}–CH_3), 1.96 (3H, s, Ac), 2.04 (3H, s, Ac), 4.55 (1H, d, J=2, C_{29}–H), 4.69 (1H, d, J=2, C_{29}–H), 4.69 (1H, d, J=10, C_3–H), 5.06 (1H, sex, J=10; 10; 4, C_2–H).

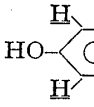
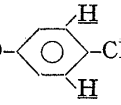
Alphitolic Acid Methyl Ester (IIb)——II (30 mg) dissolved in MeOH was methylated with CH_2N_2 at room temperature to give colorless amorphous powders of methyl ester IIb (25 mg), mp 247—249° (recrystallized from hexane), MS m/e: 486.3687 (M^+, Calcd. for $C_{31}H_{50}O_4$ 486.3709), 468 (M^+-H_2O), 450 ($M^+-2\times H_2O$), 427 ($M^+-COOCH_3$). IR ν_{max}^{KBr} cm^{-1}: 3400, 1725, 1640, 1160, 1050, 885. NMR ($CDCl_3$) δ: 0.80, 0.88, 0.91, 0.96, 1.00 (each 3H, s, CH_3), 1.69 (3H, s, C_{30}–CH_3), 2.96 (1H, d, J=10, C_3–H), 3.64 (3H, s, $COOCH_3$), 3.63 (1H, m, C_2–H), 4.58 (1H, d, J=2, C_{29}–H), 4.71 (1H, d, J=2, C_{29}–H).

Alphitolic Acid Methyl Ester Diacetate (IIc)——IIb (30 mg) was acetylated as usual way to give colorless amorphous powders of methyl ester diacetate IIc (25 mg), mp 230—234° (recrystallized from hexane), MS m/e: 570.3921 (M^+, Calcd. for $C_{35}H_{54}O_6$ 570.3920), 511 ($M^+-COOCH_3$). IR ν_{max}^{KBr} cm^{-1}: 1745, 1730, 1640, 1160, 1040, 890. NMR ($CDCl_3$) δ: 0.89, 0.92, 0.98 (15H, each s, CH_3), 1.70 (3H, s, C_{30}–CH_3), 1.98 (3H, s, Ac), 2.06 (3H, s, Ac), 3.65 (3H, s, $COOCH_3$), 4.59 (1H, d, J=2, C_{29}–H), 4.70 (1H, d, J=10, C_3–H), 4.72 (1H, d, J=2, C_{29}–H), 5.20 (1H, sex, J=10; 10; 4, C_2–H).

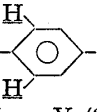
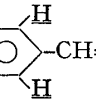
3-O-*trans*-*p*-Coumaroyl Alphitolic Acid (III)——Fraction B (9.6 g) was subjected to repeated chromatographies using C_6H_6–acetone (8: 1) as solvent to give fraction B-1, B-2 and B-3. Fraction B-2 was chromatographed over silica gel using C_6H_6–acetone (8: 1) as solvent to give colorless needles III (80 mg), mp 279—282° (recrystallized from $CHCl_3$–MeOH), $[\alpha]_D^{28}$ −33° (pyridine, c=3.75), MS m/e: 618.3933 (M^+, Calcd. for $C_{39}H_{54}O_6$ 618.3920), 572 (M^+−COOH, −H), 454 (M^+-H_2O, -*p*-coumaroyl group). IR ν_{max}^{KBr} cm^{-1}: 3420, 1705, 1690, 1640, 1635, 1610, 1590, 1050, 885. UV λ_{max}^{MeOH} nm (log ε): 312 (4.46). NMR δ: 0.94, 1.00, 1.02 (15H, each s, CH_3), 1.73 (3H, s, C_{30}–CH_3), 3.19 (1H, m, C_{19}–H), 3.96 (1H, sex, J=10; 10; 4, C_2–H), 4.63 (1H, d, J=2, C_{29}–H), 4.77 (1H, d, J=10, C_3–H), 4.79 (1H, d, J=2, C_{29}–H), 6.28 (1H, d, J=16, Ar–CH=C<u>H</u>, *trans*), 6.89 (2H, d, J=8, HO-  -CH=CH), 7.33 (2H, d, J=8, HO-  -CH=CH), 7.63 (1H, d, J=16, Ar–C<u>H</u>=CH, *trans*).

Hydrolysis of III——III (50 mg) was saponified with 2% KOH–EtOH (10 ml) for 1 hr. The reaction mixture was partitioned between EtOAc and H_2O. The EtOAc layer was evaporated to dryness and the residue was recrystallized from $CHCl_3$–MeOH to give II which was identified by direct comparison (IR and mixed melting point). The H_2O layer was neutralized with dil.HCl and extracted with EtOAc. After the evaporation of solvent the residue was recrystallized from H_2O to give colorless needles *p*-coumaric acid which was identified by direct comparison (IR and mixed melting point).

3-O-*trans*-*p*-Coumaroyl Alphitolic Acid Methyl Ester (IIIa)——III (50 mg) contaminated with a small amount of V was dissolved in MeOH–$CHCl_3$ (10 ml) and was methylated with CH_2N_2 for 2 hr at room temperature. The product was chromatographed over silica gel using C_6H_6 as solvent to afford colorless amorphous powders of methylates IIIa and Va (24 mg). NMR ($CDCl_3$) δ: 0.87—0.93 (30H, each s, CH_3), 1.64 (6H, s, C_{30}–$CH_3\times2$), 3.63 (3H, s, $COOCH_3$), 3.78 (3H, s, $COOCH_3$), 3.92 (3H, s, OCH_3), 3.98 (3H, s, OCH_3). MS m/e: 646 (M^+).

2-O-*trans*-*p*-Coumaroyl Alphitolic Acid (IV)——Fraction B-3 was subjected to the repeated chromatographies using C_6H_6–acetone (8: 1) as solvent to give colorless needles IV (26 mg), mp 279—280° (recrystallized from $CHCl_3$–MeOH), $[\alpha]_D^{28}$ −1.7° (pyridine, c=0.9). MS m/e: 618.3964 (M^+, Calcd. for $C_{39}H_{54}O_6$ 618.3920), 572 (M^+−COOH, −H), 454 (M^+-H_2O, -*p*-coumaroyl group). IR ν_{max}^{KBr} cm^{-1}: 3420, 1705, 1690, 1640, 1635, 1610, 1590, 1050, 885. UV λ_{max}^{MeOH} nm (log ε): 308 (4.95). NMR δ: 0.93, 0.99, 1.12, (15H, s, CH_3), 1.72 (3H, s, C_{30}–CH_3), 3.18 (1H, m, C_{19}–H), 3.33 (1H, d, J=10, C_3–H), 4.57 (1H, d, J=2, C_{29}–H), 4.74 (1H, d, J=2, C_{29}–H), 5.16 (1H, sex, J=10; 10; 4, C_2–H), 6.19 (1H, d, J=16, Ar–CH=C<u>H</u>, *trans*), 6.89 (2H, d, J=8, HO-  -CH=CH), 7.29 (2H, d, J=8, HO-  -CH=CH), 7.56 (1H, d, J=16, Ar–C<u>H</u>=CH, *trans*).

Hydrolysis of IV——IV was saponified as same way as that of III to give hydrolysates which were identified to be II and *p*-coumaric acid by direct comparison (IR and mixed melting point).

3-O-*cis*-*p*-Coumaroyl Alphitolic Acid (V)——Fraction B-1 was subjected to the repeated chromatographies using C_6H_6–acetone (8: 1) as solvent to give colorless needles V (50 mg), mp 208—210° (recrystallized from $CHCl_3$–MeOH). $[\alpha]_D^{24}$ +40° (pyridine, c=0.8), MS m/e: 618.3896 (M^+, Calcd. for $C_{39}H_{54}O_6$ 618.3920), 572 (M^+−COOH, −H), 454 (M^+-H_2O, -*p*-coumaroyl group). IR ν_{max}^{KBr} cm^{-1}: 3420, 1715, 1685, 1640, 1635, 1605, 1050, 885. UV λ_{max}^{MeOH} nm (log ε): 310 (4.22). NMR δ: 0.82, 0.89, 0.97, 1.00 (15H, s, CH_3), 1.75 (3H, s, C_{30}–CH_3), 3.20 (1H, m, C_{19}–H), 3.88 (1H, sex, J=10; 10; 4, C_2–H), 4.64 (1H, d, J=2, C_{29}–H), 4.66 (1H, d, J=10, C_3–H), 4.80 (1H, d, J=2, C_{29}–H), 5.83 (1H, d, J=12, Ar–CH=C<u>H</u>, *cis*), 6.82 (1H, d, J=12, Ar–C<u>H</u>=CH, *cis*), 6.88 (2H, d, J=8, HO-  -CH=CH), 7.72 (2H, d, J=8, HO-  -CH=CH).

Conversion of V to III——V (20 mg) dissolved in dioxane (10 ml) was refluxed for 24 hr. After the evaporation of the solvent the residue was recrystallized from $CHCl_3$–MeOH to give colorless needles of III (12 mg) which was identified by direct comparison (TLC and NMR).

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