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—Zizyphus 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 bilioussness, 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, 2x-hydroxyursolic acid, 2x-anthocysinic acid2) 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 Mull. we isolated three new $p$-coumaroylates of alphitolic acid. This paper concerns the structure elucidation of these esters along with known triterpenoid acids.

Fig. 1

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 : $R_1=OH, R_2=O-CO-C=C\overset{\circ}{\circ}\overset{\circ}{\circ}OH, R_3=H$
IIIa: $R_1=OH, R_2=O-CO-C=C\overset{\circ}{\circ}OMe, R_3=Me$
IV : $R_1=O-CO-C=C\overset{\circ}{\circ}OH, R_2=OH, R_3=H$
V : $R_1=OH, R_2=O-CO-C=C\overset{\circ}{\circ}OH, R_3=H$

1) Location: a) Maidashi, Higashi-ku, Fukuoka; b) Tashiro, Tosu, Saga.
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°), [α]_D^20 +6.6° (pyridine), m/e 456 (M⁺) showed blue-violet for H₂SO₄ 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 skeleton. 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°, [α]_D^20 +4.0° (pyridine), C_{20}H_{46}O_{4}, m/e 472 (M⁺), showed characteristic blue for H₂SO₄ 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 C₅-H and C₆-H at δ 3.03 and 3.72, respectively together with the signals corresponding to five tertiary methyl and isopropenyl groups. On acetylation with Ac₂O and pyridine II afforded diacetate IIa, mp 240—243°, C_{34}H_{58}O₈. The NMR spectrum of IIa exhibited two acetyl methyl and two methine proton (C₅-H and C₆-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_{56}O₄, 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_{32}H_{58}O₆, 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 C₅ and C₆ (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 aliphatic acid. Furthermore, the diequatorial 2ₜ, 3β-configuration of hydroxyl groups in II is confirmed by examination of the coupling constant (J_{2ₜ,1ₜ}=J_{3ₚ,2ₚ} =10 Hz; J_{2ₜ,1ₚ}=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 aliphatotic acid and its derivatives.\(^6\) Thus, the structure of II was determined to be aliphatic acid.

| Table I. Nuclear Magnetic Resonance Spectral Data of C₂ and C₄ Methine Protons in II, IIa, b, c, III, IV and V |
|---|---|---|---|---|---|---|
| II | IIa | IIb | IIc | III | IV | V |
| C₂ | 3.72(b) | 5.06(b) | 3.63(b) | 5.20(b) | 3.96(b) | 5.16(b) | 3.88(a) |
| | a | a | a | a | a | a | a |
| C₃ | 3.03 | a | d | a | a | a | a |
| | 4.69 | 2.96 | 4.70 | 4.77 | 3.33 | 4.66 | 3.25(b) |
| | b | b | b | b | b | b | b |
| | c | c | c | c |

a, sextet, J=10; 10 Hz b, doublet, J=10 Hz c, multiplet, d, overlapped.

The spectra were determined in CDCl₃—pyridine-d₅(10: 1)(a) or CDCl₃(b) with Me₄Si as an internal standard at 100 MHz. c) The signal is overlapped by the signal of C₄-H.

Compound C (III), mp 270—282°, [α]_D^20 -33° (pyridine), C_{29}H_{54}O₆, m/e 618 (M⁺), showed characteristic blue for H₂SO₄ and orange for benzidine reagents. III showed 3a,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 hydrogen at δ 4.66.

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

<table>
<thead>
<tr>
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<th>( \alpha )</th>
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<th>( \gamma )</th>
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<tbody>
<tr>
<td>III</td>
<td>6.28 ( (J=16) )</td>
<td>7.63 ( (J=16) )</td>
<td>7.33 ( (J=8) )</td>
<td>6.89 ( (J=8) )</td>
</tr>
<tr>
<td>IV</td>
<td>6.19 ( (J=16) )</td>
<td>7.56 ( (J=16) )</td>
<td>7.29 ( (J=8) )</td>
<td>6.89 ( (J=8) )</td>
</tr>
<tr>
<td>V</td>
<td>5.83 ( (J=12) )</td>
<td>6.82 ( (J=12) )</td>
<td>7.72 ( (J=8) )</td>
<td>6.88 ( (J=8) )</td>
</tr>
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\( R= \text{aliphatic acid} \).
The spectra were determined in CDCl\(_3\)-pyridine-d\(_6\)(10:1) with Me\(_4\)Si as an internal standard at 100 MHz.

Aromatic protons at \( \delta 6.89 \ (J=8 \text{ Hz}) \) and 7.33 \( (J=8 \text{ Hz}) \) and \( \text{trans} \) olefinic protons conjugated with aromatic ring (\( \delta 6.28 \) and 7.63, \( J=16 \text{ Hz} \)) of which a proton at \( \beta \)-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 III\( \alpha \) along with methyl ester of contaminated V. On the NMR spectrum two methoxy-carbonyl and methoxy proton signals appeared at \( \delta 3.63 \) (or 3.78) and 3.92 (or 3.98). The above results provide that III is C\(_3\) 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\(_3\) and C\(_3\)-H in II and its derivatives on the NMR spectrum. Therefore, the structure of III was established to be 3-O-\( \text{trans-} p \)-coumaroyl aliphatic acid.

Compound D(IV), mp 279—280°, [\( \alpha \)]\( \text{D} \) \( +1.7^\circ \) (pyridine), C\(_{36}\)H\(_{54}\)O\(_9\), \( m/e \) 618 \( (M^+) \), gave same positive H\(_2\)SO\(_4\) and benzidine tests to those of III. IV indicated \( \alpha,\beta \)-unsaturated carboxyl 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 (\( \delta 6.89 \) and 7.29, \( J=8 \text{ Hz} \), \( \text{trans} \) conjugated olefinic proton signals (\( \delta 6.19 \) and 7.56, \( J=16 \text{ Hz} \)) and the signals due to C\(_3\)-H and C\(_3\)-H (\( \delta 3.33 \) 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\(_3\) or C\(_3\) \( p \)-coumaroyl ester of II. The linkage of \( p \)-coumaric acid in IV was determined to be C\(_3\) position by the comparison of the chemical shifts of C\(_3\) 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-\( \text{trans-} p \)-coumaroyl aliphatic acid.

Compound E (V), mp 208—210°, [\( \alpha \)]\( \text{D} \) \( +40^\circ \) (pyridine), C\(_{39}\)H\(_{56}\)O\(_9\), \( m/e \) 618 \( (M^+) \), gave same positive H\(_2\)SO\(_4\) and benzidine tests to those of III and IV. V showed \( \alpha,\beta \)-unsaturated carboxyl 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 \( \text{cis} \) conjugated olefinic proton signals (\( \delta 5.83 \) and 6.82, \( J=12 \text{ Hz} \)) instead of those of \( \text{trans} \) in III and the proton at C\(_3\), and C\(_6\) in aromatic ring appeared in the lower-field (\( \delta 7.72 \)), 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 \( \text{cis} \) \( p \)-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 C₉ 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 C₉ position in II. Consequently, the structure of V was established to be 3-O-cis-p-coumaroyl alphatic acid. III, IV and V are the first p-coumaroylates of alphatic 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. Mass spectra were taken with a JEOH C-100H spectrometer and chemical shifts are given in δ scale with Me₄Si as an internal standard and coupling constants (J) in Hz. Abbreviations used, s, singlet; d, doublet; t, triplet; q, quartet; sex, sextet; m, multiplet; br, broad. Unless otherwise indicated solvent used was CDCl₃-pyridine-d₅ (10:1). Mass spectra were recorded on a JMS-01SG mass spectrometer with an accelerating potential of 8.3 kV, an ionizing potential of 75 eV and a source temperature of 190°. Thin-layer chromatography (TLC) was performed on Kieselgel G (Merck) using solvent system, C₆H₆-acetone (10:1) for I and derivatives of II, C₆H₆-acetone (8:1) for III—V and C₆H₆-acetone (3:1) for II. Column chromatography was performed with Kieselgel 60 (70—230 mesh) (Merck).

Isolation of Tripterpend—The dry fruits (20 kg) of Zizyphus jujuba were extracted with boiling EtOH for 1 hr, twice. The EtOH extract (11.2 kg) suspended in H₂O was extracted with BuOH. The BuOH extract (240 g) was chromatographed over silica gel using C₆H₆-acetone (7:3) as solvent to give resinous substance (112 g). The resinous substance was partitioned between 2% Na₂CO₃ and EtOAc and the EtOAc layer was evaporated to dryness. The residue (64 g) was chromatographed over silica gel using CHCl₃—EtOAc as solvent to give fraction A (25.8 g) [elution with CHCl₃—EtOAc (95:5)], fraction B (9.6 g) [elution with CHCl₃—EtOAc (85:5)] and fraction C (19.2 g) [elution with CHCl₃—EtOAc (1:1)].

Isolation of Betulic Acid (I)—Fraction A (25.8 g) was subjected to the repeated chromatographies using CHCl₃—EtOAc as solvent and the fraction eluted with CHCl₃—EtOAc (10:1) was recrystallized from CHCl₃—MeOH to give colorless needles I (968 mg), mp 293—295°, [α]D° +8.6° (pyridine, c=2.5), IR νmax cm⁻¹: 3440, 1690, 1640, 1050, 890. NMR δ: 8.03, 8.09, 1.01, 1.04 (15H, each s, CH₃), 1.73 (3H, s, C₂₉-C₂₃-H), 3.10, 3.25 (2H, m, C₂₉-H, C₂₉-H'), 4.63 (1H, d, J=2, C₂₉-H), 4.79 (1H, d, J=2, C₂₉-H), MS m/z: 456 (M⁺), 438 (M⁺—H₂O), 423, 410 (M⁺—COOH, —H).

Betulic Acid Methyl Ester (Ia) —I (70 mg) dissolved in MeOH—CHCl₃ (1:1) was methylated with CH₃ΝH₂ at room temperature to give colorless needles Ia (40 mg) (recrystallized from CHCl₃—MeOH), mp 217—218°. NMR (CDCl₃) δ: 0.75, 0.82, 0.92, 0.96 (15H, each s, CH₃), 1.69 (3H, s, C₂₉-C₂₃-H), 2.98, 3.08 (2H, m, C₂₉-H, C₂₉-H'), 4.58 (1H, d, J=2, C₂₉-H), 4.71 (1H, d, J=2, C₂₉-H). The IR and NMR spectra of Ia were superimposed with those of an authentic sample.

Isolation of Alphatic Acid (II)—Fraction C (19.2 g) was subjected to repeated chromatographies using C₆H₆-acetone as solvent and the fraction eluted with C₆H₆-acetone (3:1) was recrystallized from CHCl₃—MeOH to give colorless needles II (1.15 g), mp 275—278° (dec.). [α]D° −4.0° (pyridine, c=1.0), MS m/z: 472.5398 (M⁺, Calcd. for C₃₉H₆₆O₄ 472.3553), 454 (M⁺—H₂O), 426 (M⁺—COOH, —H). IR νmax cm⁻¹: 3400, 1690, 1640, 1050, 890. NMR δ: 0.84, 0.88, 0.97, 1.00, 1.04 (each s, CH₃), 1.74 (3H, s, C₂₉-C₂₃-H), 3.03 (1H, d, J=10, C₂₉-H), 3.72 (1H, sex, J=10; 10; 4, C₂₉-H), 4.60 (1H, d, J=2, C₂₉-H), 4.76 (1H, d, J=2, C₂₉-H).

Diacyl Alphatic Acid (Iia) —IIa (30 mg) was acetylated as usual way to give colorless amorphous powders of diacetate Ila (22.8 mg), mp 240—243° (recrystallized from EtOH), MS m/z: 556.3797 (M⁺, Calcd. for C₃₉H₆₆O₆ 556.3764), 538 (M⁺—COOH, —H). IR νmax cm⁻¹: 1735, 1695, 1640, 1260, 1040, 890. NMR (CDCl₃) δ: 0.86, 0.92, 0.96 (15H, each s, CH₃), 1.68 (3H, s, C₂₉-C₂₃-H), 1.96 (3H, s, Ac), 2.04 (3H, s, Ac), 4.55 (1H, d, J=2, C₂₉-H), 4.69 (1H, d, J=2, C₂₉-H), 4.69 (1H, d, J=10, C₂₉-H), 5.06 (1H, sex, J=10; 10; 4, C₂₉-H).

Alphatic Acid Methyl Ester (Iib) —Iib (30 mg) dissolved in MeOH was methylated with CH₃ΝH₂ at room temperature to give colorless amorphous powders of methyl ester Iib (25 mg), mp 247—249° (recrystallized from hexane), MS m/z: 486.3687 (M⁺, Calcd. for C₃₉H₆₄O₄ 486.3709), 468 (M⁺—H₂O), 450 (M⁺—2×H₂O), 427 (M⁺—COOH). IR νmax cm⁻¹: 3400, 1725, 1640, 1160, 1050, 885. NMR (CDCl₃) δ: 0.80, 0.88, 0.91, 0.96, 1.00 (each 3H, s, CH₃), 1.69 (3H, s, C₂₉-C₂₃-H), 2.06 (1H, d, J=10, C₂₉-H), 3.64 (3H, s, COOCH₃), 3.63 (1H, m, C₂₉-H), 4.58 (1H, d, J=2, C₂₉-H), 4.71 (1H, d, J=2, C₂₉-H).

Alphatic Acid Methyl Ester Diacetate (Iic) —Iic (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/z: 570.3921 (M⁺, Calcd. for C₄₀H₇₂O₄ 570.3920), 511 (M⁺—COOCH₃). IR νmax cm⁻¹: 1745, 1730, 1640, 1160, 1040, 890. NMR (CDCl₃) δ: 0.89, 0.92, 0.98 (15H, each s, CH₃), 1.70 (3H, s, C₂₉-C₂₃-H), 1.89 (3H, s, Ac), 2.06 (3H, s, Ac), 3.65 (3H, s, COOCH₃), 4.59 (1H, d, J=2, C₂₉-H), 4.70 (1H, d, J=10, C₂₉-H), 4.72 (1H, d, J=2, C₂₉-H), 5.20 (1H, sex, J=10; 10; 4, C₂₉-H).

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3-O-trans-p-Coumaroyl Alphatic Acid (III) — Fraction B (9.6 g) was subjected to repeated chromatographies using C₆H₆-acetone (8:1) as solvent to give fraction B-1, B-2 and B-3. Fraction B-2 was chromatographed over silica gel using C₆H₆-acetone (8:1) as solvent to give colorless needles III (80 mg), mp 279—282° (recrystallized from CHCl₃-MeOH, [α]D₅₀ = −33° (pyridine, ε = 2.75), MS m/z: 618.3933 (M⁺, Calcd. for C₂₉H₂₉O₅, 618.3920), 572 (M⁺—COOH, −H), 454 (M⁺—H₂O, p-coumaroyl group). IR ν max cm⁻¹: 3420, 1705, 1690, 1640, 1635, 1610, 1590, 1505, 885. UV λ max nm (log ε): 312 (4.46). NMR δ: 0.94, 1.00, 1.02 (15H, each s, CH₃), 1.73 (3H, s, C₉H₇-CH₃), 3.19 (1H, m, C₁₉-H), 3.96 (1H, sex, J = 10; 10; 4, C₆-H), 4.63 (1H, d, J = 2, C₂₉-H), 4.77 (1H, d, J = 10, C₆-H), 4.79 (1H, d, J = 2, C₂₉-H), 6.28 (1H, d, J = 16, Ar-CH=CH, trans), 6.89 (2H, H, d, J = 8, HO-CH=CH, 7.33 (2H, d, J = 8, HO-CH=CH, 7.63 (1H, d, J = 16, Ar-CH=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 H₂O. The EtOAc layer was evaporated to dryness and the residue was recrystallized from CHCl₃-MeOH to give II which was identified by direct comparison (IR and mixed melting point). The H₂O layer was neutralized with dil. HCl and extracted with EtOAc. After the evaporation of solvent the residue was recrystallized from H₂O to give colorless needles p-coumaric acid which was identified by direct comparison (IR and mixed melting point).

3-O-trans-p-Coumaroyl Alphatic Acid Methyl Ester (IIIα) — IIIα (50 mg) contaminated with a small amount of V was dissolved in MeOH–CHCl₃ (10 ml) and was methylated with CH₃JNa for 2 hr at room temperature. The product was chromatographed over silica gel using C₆H₆ as solvent to afford colorless amorphous powders of methylates IIIα and Va (24 mg). NMR (CDCl₃) δ: 0.87—0.93 (30H, each s, CH₃), 1.64 (6H, s, C₂₉-CH₃×2), 3.63 (3H, s, COOCH₃), 3.78 (3H, s, COOCH₃), 3.92 (3H, s, OCH₃), 3.98 (3H, s, OCH₃).

MS m/z: 646 (M⁺). 2-O-trans-p-Coumaroyl Alphatic Acid (IV) — Fraction B-3 was subjected to the repeated chromatographies using C₆H₆-acetone (8:1) as solvent to give colorless needles IV (26 mg), mp 279—280° (recrystallized from CHCl₃-MeOH, [α]D₅₀ = −1.7° (pyridine, ε = 0.9). MS m/z: 618.3964 (M⁺, Calcd. for C₂₉H₂₉O₅, 618.3920), 572 (M⁺—COOH, −H), 454 (M⁺—H₂O, p-coumaroyl group). IR ν max cm⁻¹: 3420, 1705, 1690, 1640, 1635, 1610, 1590, 1505, 885. UV λ max nm (log ε): 308 (4.55). NMR δ: 0.93, 0.95, 1.12, (15H, s, CH₃), 1.72 (3H, s, C₉H₇-CH₃), 3.18 (1H, m, C₁₉-H), 3.33 (1H, d, J = 10, C₆-H), 4.57 (1H, d, J = 2, C₂₉-H), 4.74 (1H, d, J = 2, C₂₉-H), 5.16 (1H, sex, J = 10; 10; 4, C₆-H), 6.19 (1H, d, J = 16, Ar-CH=CH, trans), 6.89 (2H, d, J = 8, H, H, HO-CH=CH), 7.29 (2H, d, J = 8, HO-CH=CH), 7.56 (1H, d, J = 16, Ar-CH=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 Alphatic Acid (V) — Fraction B-1 was subjected to the repeated chromatographies using C₆H₆-acetone (8:1) as solvent to give colorless needles V (50 mg), mp 208—210° (recrystallized from CHCl₃-MeOH, [α]D₅₀ = +40° (pyridine, ε = 0.8), MS m/z: 618.3896 (M⁺, Calcd. for C₂₉H₂₉O₅, 618.3920), 572 (M⁺—COOH, −H), 454 (M⁺—H₂O, p-coumaroyl group). IR ν max cm⁻¹: 3420, 1715, 1655, 1640, 1635, 1605, 1050, 885. UV λ max nm (log ε): 310 (4.22). NMR δ: 0.82, 0.89, 0.97, 1.00 (15H, s, CH₃), 1.75 (3H, s, C₉H₇-CH₃), 3.20 (1H, m, C₁₉-H), 3.88 (1H, sex, J = 10; 10; 4, C₆-H), 4.64 (1H, d, J = 2, C₂₉-H), 4.66 (1H, d, J = 10, C₆-H), 4.80 (1H, d, J = 2, C₂₉-H), 5.83 (1H, d, J = 12, Ar-CH=CH=cis), 6.82 (1H, d, J = 12, Ar-CH=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 CHCl₃-MeOH to give colorless needles of III (12 mg) which was identified by direct comparison (TLC and NMR).

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