Chemical Transformation of Embelin through Dimerization during Preparation of a Decoction

Fumiyuki KIUCHI,*[,1] Noriko SUZUKI, Yumiko FUKUMOTO, Yoshihisa GOTO, Mariko MITSUI, and Yoshisuke TSUDA

Faculty of Pharmaceutical Sciences, Kanazawa University, 13–1 Takara-machi, Kanazawa 920-0934, Japan.
Received March 11, 1998; accepted April 20, 1998

Embelin, a major constituent of *Embelia ribes* BURM. (Myrsinaceae) was transformed into different types of compounds through dimerization during preparation of a decoction. Two of the products are proposed to have furanylidene benzofuranone and 1,4-dibenzofurandione skeletons on the basis of spectroscopic means. The transformation of embelin in boiling water is markedly accelerated by the presence of fatty acids.

Key words embelin; dimerization; *Embelia ribes*; decoction; furanylidene benzofuranone; 1,4-dibenzofurandione

A number of herbal medicines are used as decoctions in traditional treatments. These decoctions, which are an extraction of the active principles with boiling or hot water, are usually prepared according to well established prescriptions and traditional procedures. During such processes, some of the constituents originally present may possibly undergo chemical changes. Hydrolytic cleavage of some glycosidic linkages and of some ester groups is commonly observed. Further transformation of the constituents, such as in the decoction of Geranium herb,[2] is also sometimes observed. Oxidation and reduction steps are less common, but are also possible. In such cases, it is important to clarify the chemical changes of the original constituents and to elucidate the actual active species in the decoction. We present here an example of an entirely different type of transformation during the preparation of a decoction: that is, a ring opening of a quinone constituent initiated by dimerization.

This finding came from the following observations. A Nepali traditional medicine called "bayubidanga" is the fruit of a Myrsinaceae plant, *Embelia* (*E.*) *ribes* BURM., which has long been known to be effective against tapeworm and applied as a decoction according to the traditional usage.[3] The active principle has been thought to be embelin (2,5-dihydroxy-3-undecyl-*p*-benzoquinone), the major ingredient of the fruit.[4] Sahu *et al.*[5] suggested that the decoction was also effective for whip-worm (*Trichuris trichiura*). During our efforts to identify the active principles for whip-worm,[6] we found that most of embelin was lost during preparation of the decoction and changed into different compounds. This paper deals with these transformations.

Results and Discussion

The fruit of *E. ribes*, when extracted with ether, gave embelin (**1**) in 2.4—4.0% yield. However, the embelin content in a decoction prepared by a traditional method[5] was *ca.* 0.03% (determined by UV). As **1** is hardly soluble in water, it was possible that **1** remained in the residual fruit. This possibility was excluded by successive extraction of the fruit with hot water and then ether: very little of **1** was detected either in the water or the ether extracts. This suggested that **1** had changed into different compound(s) on boiling with water. Thus, this transformation was examined by heating **1** in water.

Changes of Embelin in Boiling Water Embelin (**1**) is soluble in aqueous alkali and recovered unchanged on acidification even after heating, showing that it is stable in the form of a di-anion. On the contrary, it is unstable under neutral to weakly acidic conditions. Although **1** is hardly soluble in water, heating of **1** in water gave a mixture of at least four compounds (Fig. 1). The change was greatly accelerated by addition of a fatty acid such as decanoic acid. In the presence of decanoic acid, more than 90% of **1** was transformed within 1 h, and two compounds (A and B) were isolated as well characterizable products in 45.2% and 9.5% yields, respectively, after 6.5 h of heating.

Compound A (**2a**) formed fine red needles, mp 166—168 °C (from hexane), and had a molecular formula $C_{34}H_{50}O_7$ corresponding to 2×(embelin)-H_2O as suggested from its MS (M^+ 570). It was identical with the compound isolated from the hot water-treated *E. ribes* described below. In the ^{1}H-NMR spectrum, it showed two undecyl side chains [two triplet methyls at δ 0.87 and 0.88 (each 3H), and methylenes at δ 2.36 (2H, t), 2.69 (2H, t), 1.2—1.6 (36H)], and an olefinic proton at δ 7.32 as a singlet. The presence of three OH groups suggested by NMR peaks at δ 6.14, 6.84, 11.75 (disappeared on addition of D_2O) was confirmed by formation of a tri-*O*-acetate (**2b**), mp 91.5—93 °C, by usual acetylation with acetic anhydride and pyridine.

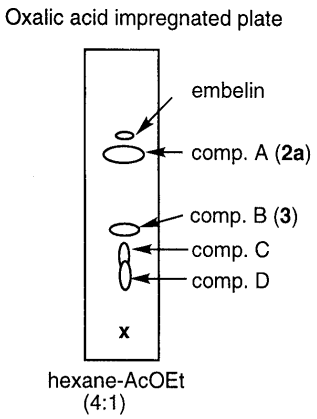

Fig. 1. Transformation of Embelin (**1**) in Boiling Water

* To whom correspondence should be addressed.

The ^{13}C-NMR spectrum showed 12 carbons (except for side chain carbons) which appeared at δ 108.4—173.3. Among those, the one at δ 108.4 which correlated with δ_H 7.32 in the C–H correlation spectroscopy (COSY) spectrum was attributable to =C–H. Four carbons at δ 107.9, 108.1, 112.0, 114.3 were assignable to C=C, four at δ 140.7, 146.8, 148.1, 149.4 to =C–O, and three at δ 162.4, 169.4, 173.3 to C=O or its equivalent. Among them, the three at δ 140.7, 146.9 and 148.1 showed long-range correlation with the proton at δ 7.32 in the heteronuclear multiple bond connectivity (HMBC) spectrum (Fig. 2). Attempts at derivatizations such as methylation and hydrogenation were fruitless.

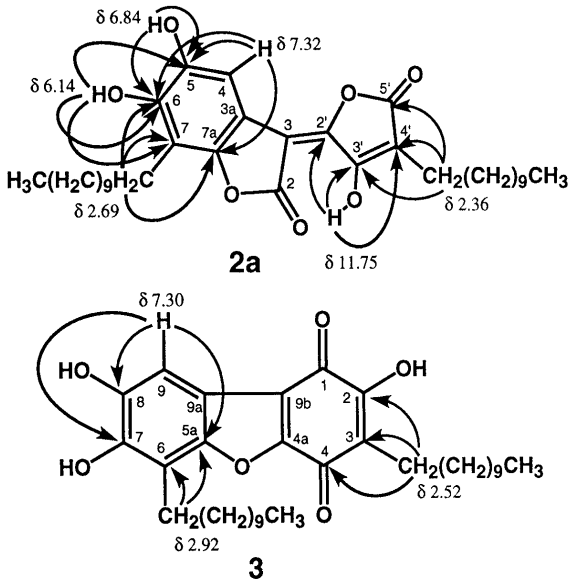

Fig. 2. HMBC (**2a**) and COLOC (**3**) Correlations

The diquinone structure **4** (Chart 1) firstly assumed was soon abandoned, because compound A exhibited IR absorptions at 1763 and 1721 cm^{-1} suggesting the presence of γ-lactone moiety. Finally, the furanylidene benzofuranone structure **2a** (in which one of the quinone rings was reduced and the other was oxidatively cleaved), similar to bovilactone-4,4 (**5**),[7] was assigned to compound A by the analysis of the product obtained from a similar reaction using 2,5-dihydroxy-3-methylbenzoquinone, in which the structure was determined by the aid of X-ray crystal analysis.[8]

Compound B (**3**) formed fine black-red crystals, mp 166—167 °C, and had a molecular formula $C_{34}H_{50}O_6$, which is one oxygen less than that of compound A. It had two undecyl side chains [δ 0.87 (3H × 2, t, Me) , 1.2—1.6 (36H), 2.52, 2.92 (each 2H, t, CH_2)], one =C–H (δ 7.30), and three OH groups (δ 5.77, 5.83, 7.06, disappeared on addition of D_2O). Among 12 carbon signals except those of the side chains, two at δ 178.9 and 180.5 were those of quinone carbonyls, five at δ 146.4, 147.2, 152.0, 152.9, 155.1 were assignable to =C–O, four at δ 114.1, 114.5, 120.2, 120.6 to C=C, and one at δ 103.2 to =C–H. These data, together with the correlation spectroscopy *via* long-range coupling (COLOC) spectrum (Fig. 2), suggested the presence of a quinone and an aromatic ring in the molecule. This compound seems to be produced

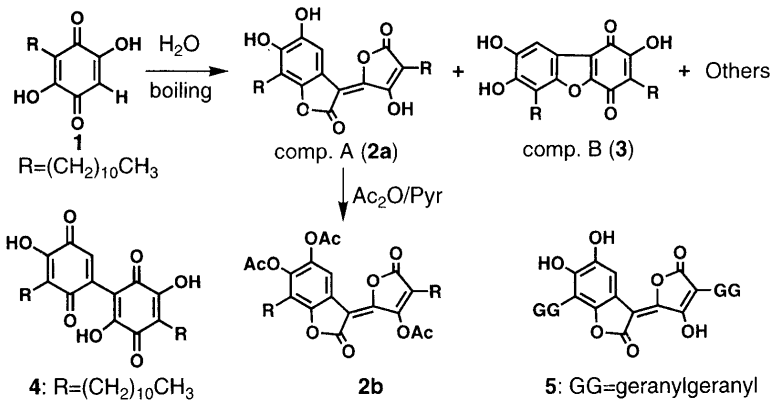

Chart 1

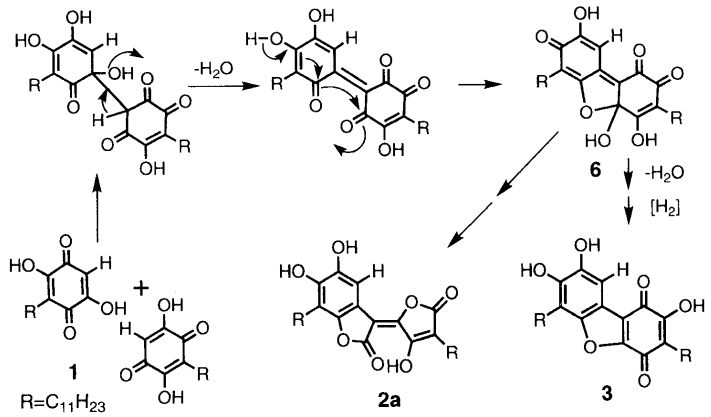

Chart 2. Reaction Path from **1** to **2a** and **3**

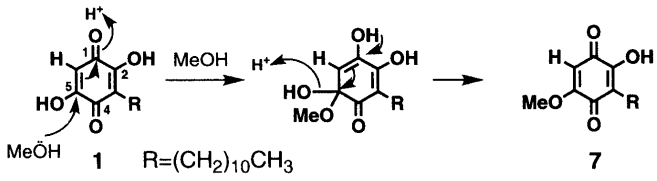

Chart 3. Formation of 5-*O*-Methylembelin (**7**)

from a common intermediate **6** (Chart 2) to **2a** by dehydration and reduction (at some stage). Thus, the 1,4-dibenzofurandione structure **3** was suggested, which is compatible with all the spectral data.[8]

The other compounds C (mp 170—180.5 °C) and D (mp 163.5—172 °C) are believed to be trimers or their degradation products, but were not fully characterized.[9]

Reaction of Embelin in Boiling Methanol Heating of embelin (**1**) in methanol under catalysis with *p*-TsOH resulted in the introduction of an *O*-methyl group giving rise to 5-*O*-methylembelin (**7**), mp 96—97 °C.[10] When decanoic acid was used instead of *p*-TsOH, **2a** (17%) was obtained along with **7** (14%). The formation of **7** can be explained by the Michael addition of methanol to the more reactive and less hindered enone system followed by elimination of water (Chart 3). 5-*O*-Methylembelin (**7**), was also obtainable by treatment of **1** with diazomethane for a short period, where the least hindered hydroxyl group was methylated.

Other Constituents of *E. ribes* The fruit of *E. ribes* contains embelin (**1**, average 3%) and a mixture of fatty acid glycerides (3—4%) which is mainly composed of linoleic acid (major) and palmitic acid (minor). When the fruit was extracted with water, the major extracts were gallic acid (*ca.* 0.1%), glucose, fructose, and glycerol. Ether extraction of the residual fruit gave fatty acids (mainly linoleic acid), which, in part, are formed from the above glycerides by hydrolysis, and a small amount of compound A (**2a**). The quantity of **1** in this extract was very small. This means that the chemical transformation of **1** in the decoction is faster than that in pure water, because there is a large amount of fatty acids (mainly linoleic acid) whose amounts were increased by hydrolytic cleavage of glycerides in the decoction. The fatty acids will assist in dissolving **1** and accelerate its transformation to various compounds including the dimers **2a** and **3**, as decanoic acid did.

Experimental

General Unless otherwise stated, the following procedures were adopted. Melting points were determined with a YANACO melting point apparatus and were uncorrected. IR spectra were measured with a Shimadzu IR-460 spectrometer using KBr disks and the data are given in cm^{-1}. UV spectra were recorded with a Shimadzu UV-1600 and given by λ_{max} in nm ($\log\varepsilon$). ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM-GSX500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) using tetramethylsilane (in $CDCl_3$ and pyridine-d_5) or trimethylsilylpropionic acid (in D_2O) as internal standards and chemical shifts are given in δ. MS were recorded with a Hitachi M-80 or JEOL JMS-SX102 and major peaks are indicated as *m/z* (%). Gas-liquid chromatography (GLC) was performed on a Shimadzu GC-4CP-PF with flame ionization detector (FID) on a 1.5% OV-1 column (2.6 mm × 1 m): carrier gas N_2, 50 ml/min. GC-MS was performed with Hitachi M-80 on an OV-1 column (3 mm × 1 m). HPLC used a TOSO CCPM system with a refractive index (RI) detector. Column chromatography was performed on acid-treated silica gel, which was prepared as follows: Wako gel C-200 was immersed in 0.5 N oxalic acid solution for 48 h, filtered, washed almost free from acid, and dried. TLC was done on Macherey–Nagel precoated plates (SIL G-25). When acid-treated plates were necessary, the plates were dipped in 0.5 N oxalic acid solution and dried before use. Visualization of spots was by spraying with 10% H_2SO_4 and heating until colors developed.

E. ribes The fruit of *E. ribes* was purchased in Kathmandzu market and supplied by the courtesy of Dr. R. B. Sahu, Institute of Medicine, Tribhuvan University, Kathmandzu, Nepal. The weight of the ether extract was 12.5—12.9% of the dried fruit. The isolated yield of embelin was variable (2.5—4.0%) depending on the lot of the sample: 2.5% (January 1990), 4.0% (July 1991), and 2.9% (January 1993). Another sample obtained in January 1993 showed 4.0%.

Constituents of *E. ribes* Ether Extraction: Fruit of *E. ribes* (the sample of July, 1991, 750 g) was pulverized and extracted 4 times with a sufficient amount of ether at room temperature with occasional stirring (each time overnight). Concentration of the combined ether extracts to *ca.* 1/4 volume gave precipitates (42.0 g) which were crystallized from acetone, then from hexane to give embelin (**1**) as orange leaflets, mp 144—145.5 °C[11] (lit. 142—143 °C)[10] (30.1 g, 4.0%). UV λ_{max}^{MeOH} ($\log\varepsilon$): 291 (4.37). On addition of 0.1% KOH (1 drop), the λ_{max} shifted to 303 nm ($\log\varepsilon$=4.30, absorption of mono-anion).[12] On further addition of 0.1% KOH, the absorption maximum shifted to longer wavelength with increasing intensity and finally showed λ_{max} at 321 nm ($\log\varepsilon$=4.46, the absorption of the di-anion). The isosbestic point between the mono-anion and di-anion was at 306.5 nm. ^{1}H-NMR: 0.88 (3H, t, J=6.6 Hz), 1.26 (16H), 1.47 (2H, m), 2.45 (2H, t, J=7.4 Hz), 6.00 (1H, s), 7.69 (2H, br s).

A portion (87 mg) of the ether extract from which embelin was removed was chromatographed on acid-treated silica gel (hexane–$CHCl_3$–AcOEt=4:1:1) to give 5 fractions: fr. 1 (7 mg, fatty acid glyceride, see below), fr. 2 (4.4 mg, embelin (**1**)), fr. 3 and fr. 4 (29.5 mg and 26.2 mg, mixtures of embelin and fatty acids), fr. 5 (6.4 mg, fatty acids). Fraction 4 was rechromatographed on normal silica gel[13] to give fatty acids (23.1 mg), which was a mixture of palmitic, linoleic, and oleic acids in a ratio of 8:11:7 (shown by GLC).

Successive Extraction with Water then Ether: The fruit (225 g) was extracted two times with hot water (each time 500 ml) for 1 h. The extract did not show the spot of embelin on TLC. The combined extract was concentrated to 500 ml and partitioned with BuOH (400 ml) and AcOH (100 ml). The upper layer (3.1 g), on concentration, gave precipitates (*ca.* 0.7 g), which were separated by filtration. The precipitates were a mixture of fructose and glucose, which were confirmed by ^{1}H- and ^{13}C-NMR, HPLC (YMC-Pack, polyamide, 75% CH_3CN, 8 ml/min: Fru, t_R=11.9 min; Glc, t_R=14.8 min), and also by GLC of the trimethylsilyl derivative (Fru, t_R=10. 67 min, Glc, t_R=15.30 min; ratio 2:1). The filtrate was chromatographed on Sephadex LH-20 eluting with water, MeOH–water (1:1), and MeOH. The first water eluate was purified by passing through a Diaion HP-20 column to yield glycerol (980 mg). The MeOH–water eluate gave gallic acid (220 mg).

Glycerol: Colorless liquid. ^{1}H-NMR (D_2O): 3.57 (2H, dd, J=11.7, 6.8 Hz), 3.65 (2H, dd, J=12.0, 4.6 Hz), 3.78 (1H, m). ^{13}C-NMR (D_2O): 62.7, 72.4. Acetylation of this sample with acetic anhydride-pyridine gave a triacetate (pale yellow liquid), which was identical with glycerol tri-*O*-acetate by ^{1}H- and ^{13}C-NMR spectra. ^{1}H-NMR ($CDCl_3$): 2.08 (6H, s), 2.10 (3H, s), 4.16 (2H, dd, J=12.2, 5.9 Hz), 4.30 (2H, dd, J=12.2, 4.4 Hz), 5.25 (1H, m). ^{13}C-NMR ($CDCl_3$): 20.6 (×2), 20.7, 62.2 (×2), 69.1, 170.0, 170.5 (×2).

Gallic Acid: Colorless crystals, mp 235—240 °C. IR: 1705. ^{1}H-NMR (D_2O): 7.10 (2H, s). ^{13}C-NMR (D_2O): 111.7, 122.8. 139.6, 146.1, 172.0. MS: 170 (M^+), 153, 125.

Part of the residual fruit (24 g) was air-dried and extracted three times with ether to give 1.92 g extract, which was chromatographed over acid-treated silica gel eluting with hexane–$CHCl_3$–AcOEt (9:0.5:0.5, 8:1:1, 6:2:2) to yield seven fractions.

Rechromatography of fr. 3 (533 mg) gave a mixture of glycerol fatty acid ester (380 mg). ^{1}H-NMR: 0.87—2.78 (characteristic of long chain fatty acids), 4.15, 4.29, 5.27 (2:2:1, similar to glycerol triacetate). Methanolysis of this fraction with 5% HCl–MeOH followed by GLC analysis showed peaks of methyl palmitate (t_R 10.57 min) and methyl linolate (t_R 12.15 min) in a ratio of 1:3. Acetylation and GLC analysis of the methanolysis product gave a peak identical with glycerol triacetate (t_R 3.55 min).

Fraction 4 (557 mg) gave, on crystallization from hexane, embelin (**1**, 76.9 mg, 0.31%). An additional crop of **1** (18.2 mg) was obtained from

the mother liquor. A part of fr. 4 (5.2 mg) in MeOH (2 ml) was treated with ethereal diazomethane and analyzed by GC-MS, which showed the presence of methyl palmitate (t_R 10.55 min, M^+ 270), methyl linolate (t_R 12.11 min, M^+ 294), and di-*O*-methylembelin (t_R 15.10 min, M^+ 322).

Fraction 5 (170 mg) gave a red spot. This compound was purified by acid-treated silica gel chromatography (hexane : $CHCl_3$: AcOEt = 8.5 : 0.75 : 0.75), crystallization from hexane, and chromatography on silica gel (hexane : AcOEt = 5 : 1) to yield compound A (**2a**, 8 mg) as fine red needles, mp 168—169 °C. This was identical with compound A prepared from embelin (**1**) as described below.

Preparation of Decoction A decoction was prepared according to the traditional method.[5] Coarse powder of the fruit of *E. ribes* (25 g) was boiled with distilled water (500 ml) until the volume was reduced to *ca.* 60 ml.

Embelin Content in Decoction Embelin content in the decoction was determined from the difference of UV absorbance at 321 nm in neutral and basic conditions. The decoction was acidified with 1 N HCl and extracted with ether. The extract was dissolved in MeOH (100 ml). The solution (1 ml) was diluted to 25 ml, whose absorbance (A) at 321 nm was 0.083. Basification of this solution with 1 N KOH increased the A at 321 nm to 0.354 ($\Delta A = 0.271$). The ΔA of an embelin solution (8.1×10^{-3} mg/ml) under the same condition was 0.703. Thus, the embelin content in the decoction from 25 g of the fruit was 7.8 mg (*ca.* 0.03%)

Stability of Embelin in Alkaline Solution A solution of embelin (102 mg) in 5% K_2CO_3 (10 ml) was heated under reflux for 12 h. Acidification and extraction of the reaction mixture recovered unchanged embelin. The TLC showed no other spot.

Reaction of Embelin in Boiling Water A mixture of embelin (**1**, 0.97 g) and decanoic acid (1.01 g) in distilled water (200 ml) was heated under reflux for 6.5 h. The cooled mixture was extracted with ether. The products were roughly separated into four fractions by chromatography on acid-treated silica gel and each fraction was purified by repeated chromatography on acid-treated silica gel, octadesyl silica gel (ODS) and/or normal silica gel to yield the following four compounds with recovery of embelin (5%) and decanoic acid (1 g).

Compound A (**2a**): Yield 438.8 mg (45.2%). Fine red needles from hexane, mp 166—168 °C. UV λ_{max}^{EtOH} (log ε): 275 (4.25), 381 (4.15), 466 (4.07). IR: 3320, 1763, 1721, 1651, 1626, 1463. ^{1}H-NMR ($CDCl_3$): 0.87, 0.88 (each 3H, t, J = 7.1 Hz, Me), 1.2—1.4 (32H), 1.58 (4H, m), 2.36 (2H, t, J = 7.6 Hz, 4′-CH_2), 2.69 (2H, t, J = 7.6 Hz, 7-CH_2), 6.14 (1H, br s, 6-OH), 6.84 (1H, br s, 5-OH), 7.32 (1H, s, H-4), 11.75 (1H, s, 3′-OH). ^{13}C-NMR ($CDCl_3$): 14.1—31.9 (side chain carbons), 107.9, 108.1 (C-3 or 3a and C-4′), 108.4 (C-4), 112.0 (C-3 or 3a), 114.3 (C-7), 140.7 (C-5), 146.8 (C-6), 148.1 (C-7a), 149.4 (C-2′), 162.4 (C-3′), 169.4 (C-5′), 173.3 (C-2). MS: 570 (M^+, 66), 429 (6), 346 (19), 289 (12), 205 (14). *Anal.* Calcd for $C_{34}H_{50}O_7$: C, 71.55; H, 8.83. Found: C, 71.68; H. 8.94.

Compound B (**3**): Yield 92.2 mg (9.5%). Fine black-red crystals from hexane, mp 166—167 °C. UV λ_{max}^{EtOH} (log ε): 214 (4.56), 259 (4.45), 290 (4.13), 356 (3.82), 551 (3.40). IR: 1661, 1639, 1618. ^{1}H-NMR: 0.87 (3H × 2, t, J = 6.8 Hz, Me), 1.2—1.4 (34H), 1.50 (2H, m), 2.52 (2H, t, J = 7.5 Hz, 3-CH_2), 2.92 (2H, t, J = 7.5 Hz, 6-CH_2), 5.77, 5.83, 7.06 (each 1H, s, OH). 7.30 (1H, s, H-9). ^{13}C-NMR (CD_3OD): 14—33 (side chain carbons), 103.2 (C-9), 114.1 (C-6), 114.5 (C-9a), 120.2 (C-9b), 120.6 (C-3), 146.4, 147.2 (C-7 and C-8), 152.0 (C-5a), 152.9 (C-4a), 155.1 (C-2), 178.9 (C-4), 180.5 (C-1). MS: 554 (M^+, 100), 414 (26), 273 (17). *Anal.* Calcd for $C_{34}H_{50}O_6$: C, 73.61; H, 9.09. Found: C, 73.49; H, 9.41.

Compound C: Yield 22 mg (*ca.* 2.3%). Red powder, mp 170—180.5 °C. ^{1}H-NMR (CD_3OD): 0.89 (3H × 3, m), 1.3—1.7 (m), 2.47 (2H, t, J = 7.6 Hz), 2.60 (2H, m), 2.88 (2H, t, J = 7.6 Hz). 6.41 (1H, s). MS: 570 (8), 531 (21), 501 (16), 332 (14), 280 (15), 139 (18).

Compound D: Yield 32.1 mg (*ca.* 3.3%). Black powder from hexane, mp 163.5—172 °C. ^{1}H-NMR ($CDCl_3$): 0.87 (3H × 3), 1.2—1.7 (56H), 2.44 (2H, m) 2.55 (2H, t, J = 7.5 Hz), 2.76 (2H, m), 5.43 (1H, s, OH), 5.70 (1H, br s, OH), 6.10 (1H, s), 6.92 (1H, s, OH), 7.50 (1H, br s, OH), 7.68 (1H, s, OH). MS: 834 (2), 805 (12), 664 (6), 583 (3), 555 (12), 414 (3), 386 (3), 294 (4), 280 (25), 214 (9), 139 (27), 73 (30), 43 (100).

Acetylation of Compound A (2a) Compound A (**2a**, 63.7 mg) was acetylated with Ac_2O (1 ml) and pyridine (1 ml) for 70 h at room temperature and worked up as usual (ether extraction). Chromatography of the product on normal silica gel column gave a yellow compound (46.3 mg) which was crystallized from MeOH to give a triacetate **2b** (17.6 mg, 22.6%) as yellow needles, mp 91.5—93 °C. ^{1}H-NMR ($CDCl_3$): 0.88 (6H, br t, J = 6.7 Hz), 1.2—1.4 (30H), 1.55 (6H, m), 2.30, 2.33, 2.46 (each 3H, s, Ac), 2.40 (2H, t, J = 7.6 Hz), 2.56 (2H, t, J = 7.8 Hz), 7.63 (1H, s, Ar-H). ^{13}C-NMR ($CDCl_3$): 106.4, 118.3, 119.7, 121.0, 127.5, 139.6, 143.5, 150.0, 150.3, 154.8, 164.8, 165.2, 167.5, 167.8, 168.4, and signals at 14.1—31.9 due to side chains and acetyl methyls. MS: 696 (M^+, 6), 654 (32), 612 (84), 570 (100). HR-MS: Calcd for $C_{40}H_{56}O_{10}$: 696.3873. Found: 696.3863.

Changes of Embelin in Boiling Methanol A mixture of **1** (500 mg) and decanoic acid (440 mg) in MeOH (15 ml) was heated under reflux for 25 h. Evaporation of the solvent and crystallization of the residue from hexane gave recovered embelin (354 mg). Chromatography of the mother liquor on acid-treated silica gel with hexane–AcOEt gave **2a** (38 mg) and then 5-*O*-methylembelin (**7**, 48 mg) from the 4 : 1 eluate. Recrystallization of each fraction from hexane gave **2a** (23.6 mg, 4.9%; net yield, 16.7%) and **7** (22.1 mg, 4.2%; net yield, 14.4%).

5-*O*-Methylembelin (**7**): Yellow needles from hexane, mp 96—97 °C (lit. 95—96 °C).[10] IR: 3350, 1633, 1600. ^{1}H-NMR ($CDCl_3$): 0.87 (3H, t, J = 7.1 Hz), 1.25—1.29 (16H), 1.45 (2H, m), 2.43 (2H, t, J = 7.6 Hz), 3.85 (3H, s, OMe), 5.83 (1H, s), 7.22 (1H, s, OH). ^{13}C-NMR ($CDCl_3$): 14.1, 22.6, 22.7, 28.0, 29.3, 29.4, 29.55 × 2, 29.60, 29.64, 31.9, 56.7, 102.1, 119.2, 151.5, 161.1, 181.7, 182.8. MS: 308 (M^+, 22), 221 (24), 181 (100), 168 (37). *Anal.* Calcd for $C_{18}H_{28}O_4$: C, 70.10; H, 9.15. Found: C, 70.20; H, 9.19.

Methylation of Embelin with Diazomethane Embelin (**1**, 100 mg) was suspended in ether (5 ml) and treated with ethereal diazomethane for 5 min. Filtration of the reaction mixture and chromatography of the filtrate in ether on normal silica gel gave 5-*O*-methylembelin (**7**, 30 mg), mp 96—97 °C.

Acknowledgements The authors thank Dr. R. B. Sahu of Tribhuvan University, Nepal, for providing samples of *E. ribes*. This research was supported in part by a Grant-in-Aid for Scientific Research (No. 04670226 and 07672263) from the Ministry of Education, Science, Sports and Culture of Japan.

References and Notes

1) Present address: *Graduate School of Pharmaceutical Sciences, Kyoto University, Yoshida, Sakyo-ku, Kyoto 606–8501, Japan.*
2) Okuda T., Yoshida T., Mori K., Hatano T., *Heterocycles*, **15**, 1323—1348 (1981); Okuda T., Mori K., Ishino M., *Yakugaku Zasshi*, **99**, 505—509 (1979); Okuda T., Yoshida T., Nayeshiro H., *Chem. Pharm. Bull.*, **25**, 1862—1869 (1977).
3) Jayaweera D. M. A. (ed.), "Medicinal Plants Used in Ceylon," Part IV, The National Science Council of Sri Lanka, Colombo, 1981, p. 111; Sala A. V. (ed.), "Indian Medicinal Plants," Vol. 2, Orient Longman, Madras, 1993, p. 368.
4) Thompson R. H., "Naturally Occurring Quinones," Academic Press, New York, 1971, p. 137.
5) Sahu R. B., "Collected Papers on the Control of Soil-transmitted Helminthiases," Vol. IV, 1989, pp. 82—89; *idem, ibid.*, Vol. V, 1993, pp. 168—173.
6) Tsuda Y., Suzuki N., Kiuchi F., Kondo K., Akao N., *Jpn. J. Parasitol.*, **45**, 491—497 (1996).
7) Jägers E., Steglich W., *Angew. Chem. Int. Ed. Engl.*, **20**, 1016—1017 (1981).
8) Details of the reaction mechanism together with rigorous structure determination will be discussed in an accompanying paper (Kiuchi F., Takashima H., Tsuda Y., *Chem. Pharm. Bull.*, **46**, 1229—1234 (1998).
9) The major difficulty of their characterization is due to the fact that the original embelin is contaminated, even after repeated crystallization, with a CH_2CH_2 higher homologue (see reference 11); this fact amplifies the complexity in dimers, and more so in trimers, thus implying the necessity of carrying out an analogous reaction with use of a homogeneous model compound.
10) Joshi B. S., Kamat V. N., *J. Chem. Soc., Perkin Trans. 1*, **1975**, 327—332.
11) This sample contained a higher alkyl homologue as shown by MS ion monitoring method: m/z 294 [R = $(CH_2)_{10}CH_3$] was 96.16%, m/z 322 [R = $(CH_2)_{12}CH_3$] was 3.84%.
12) A derivative of mono-anion (mono-potassium embelate) was actually isolated as fine red-purple crystals, mp > 300 °C, which showed λ_{max} at 300 nm (4.19) (reference 5). This compound regenerated embelin on acidification.
13) Embelin was not eluted from a normal silica gel column, but was eluted with good recovery from an acid-treated silica gel column.