Flavone and Triterpenoid Constituents of *Elaegia utilis*

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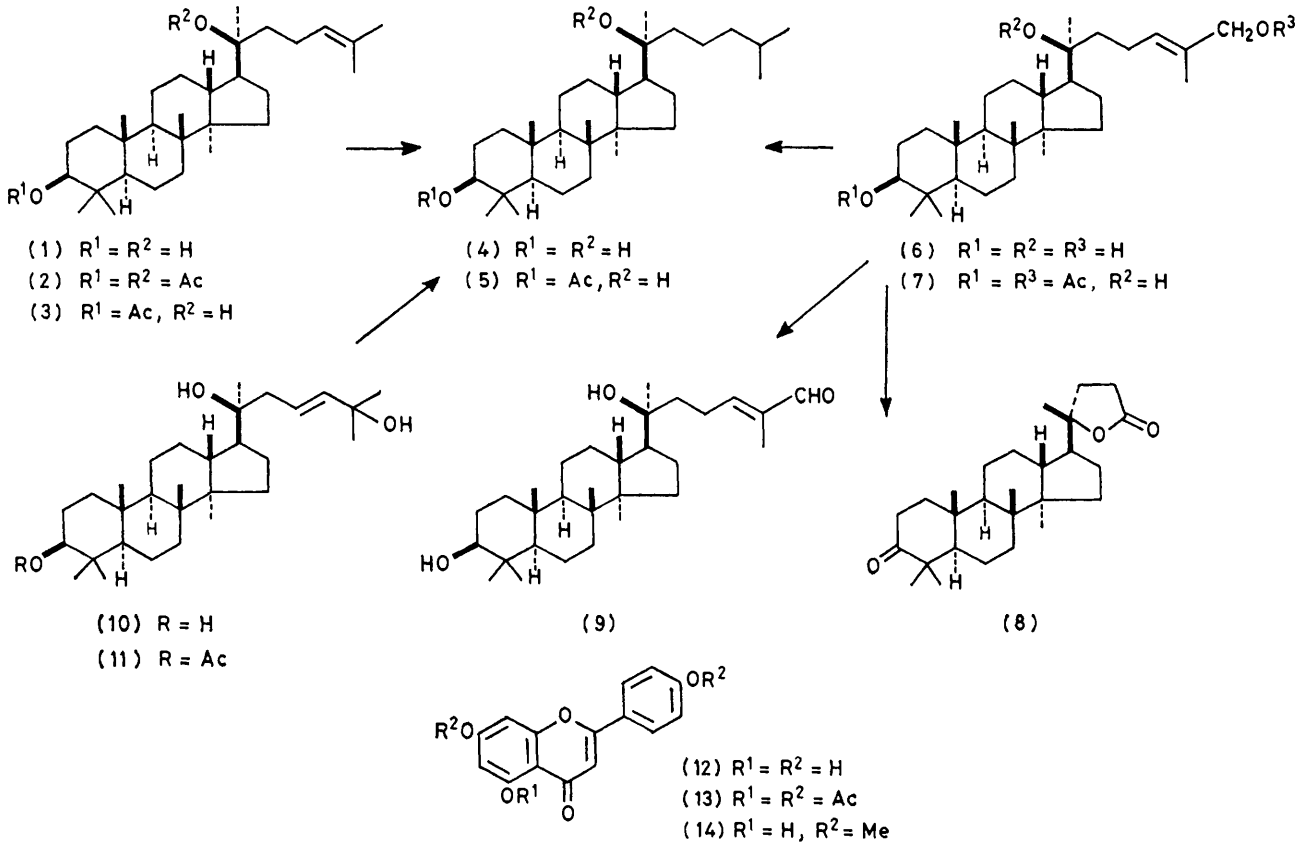
By neutral solvent extraction of *Elaegia utilis* resin, there have been isolated the flavone apigenin (12) and the tetracyclic triterpenoid isofouquierol (10), whose structure has been confirmed. Dammarenediol-II (1) (in dimorphic form) and a new triterpenoid, (20*S*)-dammar-24-ene-3β,20,26-triol (6), were obtained from the neutral non-saponifiable fraction.

THE buds of *Elaegia utilis*, found in the Departamento of Nariño, Colombia, yield a resin, known as Barniz de Pasto, used as an adhesive, a fine translucent wood finish, and a protective coating for paintings.[1] We report here the isolation and identification of constituents isolated by neutral solvent extraction and from the non-saponifiable fraction.



The resin was successively extracted with benzene, acetone, and methanol and the insoluble residue saponified. From the neutral non-saponifiable fraction were obtained two products (A and B), readily separated by silica gel thin-layer or dry column chromatography.[2]

Constituent A was identified as a low-melting modification of (20*S*)-dammar-24-ene-3β,20-diol (dammarene-diol-II) (1), previously isolated from dammar resin.[3,4] Although it was obtained crystalline from aqueous ethanol, and with consonant formula $C_{30}H_{52}O_2$ from element analysis, immediate identification of this product as (1) was precluded by several apparent discrepancies. Thus, the m.p. (75—79°) was incompatible with that reported (130—134°) by several groups,[3,5,6] the mass spectrum did not reveal the corresponding molecular ion, but instead the $M - H_2O^+$ parent peak[7] and characterization by acetylation yielded in addition to a monoacetate [with constants in excellent agreement with those reported for dammerenediol-II monoacetate (3)], a diacetate $C_{34}H_{56}O_4$ hitherto unreported. The n.m.r. spectra[8-10] of constituent A and

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the two acetate derivatives, however, were consistent with formulations (1)—(3). In addition, catalytic hydrogenation of the diol (1) and monoacetate (3) yielded respectively the known dihydro-products (4) and (5). The possibility that the low m.p. of (1) might be attributable to mixed crystal formation with an unidentified impurity was considered unlikely, since (1) gave the monoacetate derivative in *ca.* 98% yield of isolated product when the acetylation was conducted at room temperature. The dimorphic nature of dammarenediol-II was finally established by crystallization interchangeably from aqueous ethanol or nitromethane to yield the respective lower- and higher-melting forms. An alcohol [flakes, m.p. 78.5—82° (from methanol)] obtained by sodium borohydride reduction of dipterocarpol [11] is presumably the same form of dammarenediol-II as reported here.

Constituent B had the molecular formula $C_{30}H_{52}O_3$ and gave a negative tetranitromethane unsaturation test, although the n.m.r. spectrum indicated one vinyl proton. On acetylation at room temperature, it yielded a diacetate which gave a yellow colour with tetranitromethane. From this it was concluded that constituent B possessed an allylic alcohol function. Of more import, on catalytic hydrogenation, it gave in excellent yield (20*S*)-dammarane-3β,20-diol (4). On oxidation with Jones reagent, constituent B yielded the known [3,4,12] oxo-trinorlactone (8), thus restricting placement of the allylic alcohol functionality to the terminus of the dammarane side chain. With this structure limitation, we favour formulation of constituent B as (20*S*)-dammar-24-ene-3β,20,26-triol (6). Since the hydrogenation evidence established the presence of secondary and tertiary alcohol functions, a two-proton multiplet shown by the triol at δ 4.20 in the n.m.r. spectrum (shifted to δ 4.47 in that of the diacetate) supports the conclusion that the remaining hydroxy-function is primary. In additional support, oxidation of the triol with manganese dioxide in tetrahydrofuran yielded a conjugated aldehyde (9). An assignment of configuration to the double bond is not totally unequivocal. We favour the *trans*(*E*)-configuration for the parent triol [as in formula (6)], since the low field chemical shift value of the olefinic proton in the derived aldehyde (9) suggests strong deshielding by the carbonyl function. The aldehyde does, however, have a rather low u.v. molar extinction coefficient; a similar situation pertains with masticadienonic acid.[13,14]

From the acetone-soluble fraction, two products (C and D) were readily separated and identified. The empirical constants and n.m.r. spectrum of constituent C corresponded closely to those reported for isofouquierol, recently isolated from *Fouquiera splendens* Engelm and for which the structure (10), on the basis of the n.m.r. spectrum and without configuration assignment, was suggested.[15] This identification was confirmed by formation of the monoacetate (11) with concordant data. Since we have also converted (10) by catalytic hydrogenation into (20*S*)-dammarane-3β,20-diol (4), this establishes isofouquierol as (20*S*)-dammar-23-ene-3β,20,25-triol.

Constituent D was obtained as a yellow solid whose mass spectrum (M^+ 270) indicated the molecular formula $C_{15}H_{10}O_5$. With diazomethane, it yielded a dimethyl ether (M^+ 298) and with pyridine–acetic anhydride a triacetate (M^+ 396). The n.m.r. spectra of constituent D and derivatives were consistent with formulation of the former as 4′,5,7-trihydroxyflavone (apigenin) (12), and this was confirmed by direct comparison with an authentic specimen and derivatives. The n.m.r. and mass spectra of apigenin and derivatives are readily interpreted from the existing data available from general reviews of flavonoid compounds.[16]

From the benzene-soluble fraction, dammarenediol-II (1) was obtained by t.l.c.

EXPERIMENTAL

N.m.r. spectra were determined for solutions in [^{2}H]-chloroform (unless otherwise stated) with tetramethylsilane as internal standard. Analytical and preparative t.l.c. procedures were carried out on silica gel 60PF-254 + 366 (Merck) and silica gel 60 (70—230 mesh) (Merck), respectively. M.p.s were determined with either a Gallenkamp or a Fisher–Johns apparatus. Specific rotations were determined for solutions in chloroform.

Fractionation of Elaegia utilis *Resin.*—The hard resin (128 g) was extracted with benzene for 2 days to yield a green viscous oil (2.5 g, 2%), then with acetone for the same time to give a greenish gummy solid (9.0 g, 7%). The insoluble fraction was then ground to a fine powder and re-extracted with methanol to give an oil (2.5 g, 2%).

The residue was heated under reflux with 50% aqueous methanol (600 ml) containing potassium hydroxide (30 g) for 2 h, cooled, diluted with water (600 ml), and extracted with ether (3 × 250 ml). Evaporation of the washed and dried (Na_2SO_4) extract yielded the neutral non-saponifiable fraction as a white solid (9.2 g; m.p. 100—150°). Acidification of the aqueous phase precipitated a less dense oil, which on separation solidified after 2—3 days to a brown powder (*ca.* 70%).

Isolation of Constituents A *and* B *from the Neutral Non-saponifiable Fraction.*—(a) *Thin-layer chromatography.* A solution of the solid (100 mg) in tetrahydrofuran (*ca.* 1 ml) was applied as a band to a silica gel G plate (1 mm; 20 × 20 cm) and developed with benzene–ether (3 : 1). Treatment with iodine revealed two zones (R_F 0.3—0.5 and 0.0—0.2), each of which was extracted with ether. Solvent removal from the higher R_F zone gave constituent A (52 mg; m.p. 75° after one crystallization from aqueous ethanol) and from the lower R_F zone constituent B (40 mg; m.p. 196° from ethanol).

(b) *Dry column chromatography.* Silica gel (100 g) was dried at 110 °C for 24 h, then deactivated by addition of water (12 ml). To a solution of the neutral fraction (20 g)

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in tetrahydrofuran (50 ml), silica gel (20—30 g) was added, and the solvent was removed under reduced pressure. The resultant free-flowing solid was added to the top of a column of silica gel (25 × 2 in diam.) which was then percolated with benzene–ether (85 : 15). The first 150 ml of eluate contained no product, and the subsequent 2 500 ml was evaporated to give constituent A [11.5 g; homogeneous by t.l.c.; R_F 0.60 in benzene–acetonitrile (4 : 1)]. Elution with methanol (2 000 ml) then yielded constituent B (8.1 g; homogeneous by t.l.c., R_F 0.30 in the same solvent system).

Identification of Constituent A *as Dammarenediol*-II [(20*S*)-*Dammar*-24-*ene*-3β,20-*diol*] (1).—(*a*) Crystallization of constituent A from aqueous ethanol gave long needles, m.p. 75—76°, $[\alpha]_D$ +34° (*c* 0.9), ν 3 350 cm^{-1}, yellow colour with tetranitromethane [Found: C, 81.1; H, 11.8%; *m/e*, 426.387 5. Calc. for $C_{30}H_{52}O_2$: C, 81.0; H, 11.8%. Calc. for $C_{30}H_{50}O$ $(M - H_2O)^+$: *m/e* 426.386 2]; δ 0.77, 0.84, 0.87, 0.97(2), and 1.14 (six tert. Me), 3.19 (m, 3α-H), and 5.07 (m, H-24).

(*b*) *Acetylation at* 100 °C. Constituent A (2.01 g), pyridine (50 ml), and acetic anhydride (50 ml) were heated on a steam-bath for 24 h. Work-up in the usual way gave a solid product [2.21 g; R_F 0.26 and 0.47 in benzene–ether (2 : 1)]. A solution of the product (850 mg) in light petroleum (100 ml) was chromatographed on alumina (25 × 2.5 cm) eluted successively with light petroleum (400 ml), light petroleum–benzene (1 : 1; 200 ml), benzene (300 ml), benzene–ether (9 : 1; 200 ml), and benzene–ether (4 : 1; 500 ml). After the first 860 ml, the next 170 ml gave (20*S*)-*dammar*-24-*ene*-3β,20-*diol diacetate* (2) as prisms (410 mg), m.p. 148—150°, $[\alpha]_D$ +42° (*c* 0.5) (Found: C, 77.2; H, 10.7. $C_{34}H_{56}O_4$ requires C, 77.4; H, 10.5%); δ 0.86 (s, 4α- and 4β-Me), 0.88 (s, 10β- and 14α-Me), 0.98 (s, 8β-Me), 1.38 (s, 20-Me), 1.62 (m, 26- and 27-H_3), 1.95 (s, 20-OAc), 2.03 (s, 3β-OAc), 4.51 (m, 3α-H), and 5.08 (m, H-24). The last 130 ml yielded the 3-monoacetate (3) (440 mg), m.p. 136—137°, $[\alpha]_D$ +38° (*c* 0.5), as flakes (from methanol) (lit.[3] for dammarenediol-II monoacetate, m.p. 135—137°, $[\alpha]_D$ +37°); δ 0.86 (s, 4α- and 4β-Me), 0.88 (s, 10β- and 14α-Me), 0.98 (s, 8β-Me), 1.14 (s, 20-Me), 1.62 (m, 26- and 27-H_3), 2.03 (s, 3β-OAc), 4.49 (m, 3α-H), and 5.09 m (H-24).

(*c*) *Acetylation at room temp.* Constituent A (1.1 g) in pyridine–acetic anhydride was kept overnight at room temp. Work-up as in (*b*) gave dammarenediol-II monoacetate (3) as flakes (1.1 g), m.p. 136—137°. A solution of (3) (151 mg) in methanol (15 ml) was stirred overnight with aqueous 20% potassium hydroxide (20 ml); the product was worked up and crystallized from methanol and nitromethane to give dammarenediol-II (1) as short prisms, m.p. 132—133°, $[\alpha]_D$ +33° (*c* 0.2) (lit.,[4] m.p. 131—133°, $[\alpha]_D$ +33°); after recrystallization from aqueous ethanol the m.p. was 77—79°.

(*d*) *Catalytic hydrogenation.* To a solution of constituent A (430 mg) in ethanol (25 ml) was added palladium–carbon (10%; 100 mg), and the mixture was stirred under hydrogen at room temp. for 1 h. Removal of catalyst and solvent and crystallization of the solid residue from nitromethane gave (20*S*)-dammarane-3β,20-diol (4) as small prisms, m.p. 131—133°, $[\alpha]_D$ +36° (*c* 0.5) (lit.[4] for dammaranediol-II, m.p. 133—135°, $[\alpha]_D$ +35°). Similar hydrogenation of constituent A monoacetate yielded the 3-monoacetate (5) as needles, m.p. 106—108°, $[\alpha]_D$ +40° (*c* 0.62) (lit.[4] for dammaranediol-II monoacetate, m.p. 106—108°, $[\alpha]_D$ +41°).

Conversion of (20S)-*Dammar*-24-*ene*-3β,20-*diol Diacetate* (2) *into Dammarenediol*-II (1).—Lithium aluminium hydride (250 mg) was added to a solution of the diacetate (42 mg) in ether (10 ml). The mixture was refluxed for 4 h, then worked up in the usual way to give dammarenediol-II (31 mg), m.p. 132—133° (from nitromethane).

Identification of Constituent B *as* (20S)-*Dammar*-24-*ene*-3β,20,26-*triol* (6).—Recrystallization of constituent B from ethanol gave the *triol* (6) as glistening plates, m.p. 198—199°, $[\alpha]_D$ ±0° (*c* 0.26), ν 3 350 cm^{-1}, giving a negative tetranitromethane test [Found: C, 78.5; H, 11.6%; *m/e*, 442.379 7. $C_{30}H_{52}O_3$ requires C, 78.2; H, 11.4%. $C_{30}H_{50}O_2$ $(M - H_2O)^+$ requires *m/e*, 442.381 1]; δ ([$^2H_5$]pyridine) 0.87, 0.95, 1.00(2), 1.18, and 1.35 (six tert. Me), 1.80 (s, 27-H_3), 3.30 (m, H-3α), 4.20 (2 H, m, H-26), and 5.75 (m, H-24).

Dammar-24-*ene*-3β,20,26-*triol* 3β,26-*Diacetate* (7).—Acetylation of the triol (5) (100 mg) in pyridine–acetic anhydride at room temp. overnight gave the *triol diacetate* (7), m.p. 110—112°, $[\alpha]_D$ +16° (*c* 0.9) as short needles from methanol. It gave a yellow colour with tetranitromethane (Found: C, 75.15; H, 10.45. $C_{34}H_{56}O_5$ requires C, 74.95; H, 10.4%); δ 0.86 (s, 4α- and 4β-Me), 0.88 (s, 10β- and 14α-Me), 0.98 (s, 8β-Me), 1.15 (s, 20-Me), 1.71 (s, 27-H_3), 2.03 (s, OAc), 2.06 (s, OAc), 4.47 (m, 3α-H), 4.47 (2 H, m, H-26), and 5.48 (m, H-24).

Catalytic Hydrogenation of Constituent B.—A solution of the triol (6) (195 mg) in ethanol (40 ml) was stirred with palladium–carbon (5%; 40 mg) under hydrogen at room temp. Crystallization of the solid residue (196 mg) obtained by filtration and evaporation gave (20*S*)-dammarane-3β,20-diol (4) (100 mg), m.p. 132—133°, $[\alpha]_D$ +32° (*c* 0.4) (identical by t.l.c., i.r., and n.m.r.).

Oxidation of Constituent B *to the Oxo-trinorlactone* (8).—To a solution of the triol (6) (200 mg) in acetone (50 ml) was added Jones reagent (2 ml; 1.4M). After 2 h the mixture was filtered, diluted with water (50 ml), and extracted with ether (3 × 40 ml). Evaporation of the washed and dried extract gave a solid (192 mg; m.p. 52—56°, showing 2 principal spots on t.l.c., R_F 0.27 and 0.75 in $CHCl_3$). Preparative t.l.c. and isolation of the product with lower R_F value gave the oxo-trinorlactone (8), m.p. 182—184°, $[\alpha]_D$ +69° (*c* 0.93), $\bar{\nu}$ 1 775 and 1 705 cm^{-1} (Found: M^+, 414.314 6. Calc. for $C_{22}H_{42}O_3$: *M*, 414.313 4); n.m.r. spectrum as reported.[11]

(20S)-3β,20-*Dihydroxydammar*-24-*en*-26-*al* (9).—Manganese dioxide (2.0 g) was added to a solution of the triol (6) (206 mg) in dry tetrahydrofuran (30 ml). The mixture was stirred at room temp. overnight, then filtered and evaporated to give the *aldehyde* (9) as a solid foam, homogeneous by t.l.c., but not obtained crystalline from common solvents (Found: C, 78.2; H, 10.8. $C_{30}H_{50}O_3$ requires C, 78.55; H, 11.0%); δ 0.77 (s, 4α-Me), 0.85 (s, 4β-Me), 0.88 (s, 14α-Me), 0.97 (8β- and 10β-Me), 1.15 (s, 20-Me), 2.20 (s, 27-H_3), 3.18 (m, 3α-H), 6.52 (m, H-24), and 9.45 (s, H-26), $\bar{\nu}$ 1 680 and 1 600 cm^{-1}; $\lambda_{max.}$ 228 nm (ε 5 600).

Isolation of Constituents C *and* D *from the Acetone-soluble Fraction.*—The greenish gummy solid (7.05 g) was dissolved in ether–benzene (1 : 1) and filtered through a column (4 × 1.5 in diam.) of Florisil (Fisher; 100—200 mesh) to remove pigments. Elution with the same solvent (2 l) and evaporation gave a residual yellow powder (4.2 g), and with methanol–water (4 : 1) an acidic fraction as a brown viscous oil (1.95 g).

(a) *Dry column chromatography.* A solid suspension of

the yellow powder (1.0 g) on silica gel (10 g) was added to the top of a dry column (7 × 1.5 in diam.) of silica gel, which was then percolated with benzene–ether (3 : 2). The first 100 ml of eluate yielded constituent C (95 mg); the next 140 ml yielded no residue; and the subsequent 150 ml yielded constituent D (130 mg).

(b) *Without chromatography*. The yellow powder (2.0 g) was added to a solution of potassium carbonate (1.2 g) in water (30 ml). The mixture was stirred at room temperature for 3 h, then diluted with water (100 ml), and extracted with ether (3 × 50 ml). Evaporation of the dried extract yielded constituent C (205 mg). Addition of acetic acid (4 ml) to the aqueous fraction precipitated constituent D as a yellow solid (406 mg), collected by filtration. Further acidification to pH 3 gave a precipitate (460 mg) of an acid mixture.

Identification of Constituent C *as* (20S)-*Trihydroxydammar*-23-*ene*-3β,20,25-*triol* (*Isofouquierol*) (10). After isolation as above, constituent C was crystallized once from nitromethane and methanol to give isofouquietol (10) as prisms, m.p. 101—103°, $[\alpha]_D$ +22° (*c* 1.1) (lit.,[15] m.p. 108°, $[\alpha]_D$ +24°), n.m.r. spectrum identical with that reported. On acetylation, it yielded a monoacetate (11), m.p. 134—136°, $[\alpha]_D$ +34° (*c* 0.67) (lit.,[15] m.p. 137—140°, $[\alpha]_D$ +37°).

The triol (10) (12 mg) was stirred in ethanol (20 ml) under hydrogen with palladium–carbon (5%; 20 mg) for 4 h. Filtration, evaporation, and crystallization of the residue from nitromethane gave (20*S*)-dammarane-3β,20-diol (4) (6 mg), m.p. and mixed m.p. 130—132°.

Identification of Constituent D *as* 4′,5,7-*Trihydroxyflavone* (*Apigenin*) (12).—Crystallization of constituent D from acetone gave apigenin (12) as yellow needles, m.p. 351—354° (decomp.) (lit.,[17] 348—350°); δ[$(CD_3)_2SO$] 6.30 (d, *J* 2 Hz, H-6), 6.51 (d, *J* 2 Hz, H-8), 6.71 (s, H-3), 7.02 (d, *J* 9 Hz, H-3′ and -5′), 7.91 (d, *J* 9 Hz, H-2′ and -6′), and 13.08 (s, 5-OH); *m/e* (*cf.* ref. 18) 270 (M^+, 100%), 242 (38), 152 (34), 151 (38), 124 (25), 121 (50), and 118 (31); $\bar{\nu}$ (*cf.* ref. 19) (KBr) *ca.* 3 000, 1 655, 1 610, 1 505, 1 360, and 837 cm^{-1}.

Apigenin triacetate (13), obtained from (12) with pyridine–acetic anhydride overnight and crystallized from methanol, had m.p. 179—181° (lit.,[17] 181—182°); δ 2.33 (s, 4′- and 7-OAc), 2.42 (s, 5-OAc), 6.62 (s, H-3), 6.86 (d, *J* 2 Hz, H-6), 7.24 (d, *J* 9 Hz, H-3′ and -5′), 7.35 (d, *J* 2 Hz, H-8), and 7.87 (d, *J* 9 Hz, H-2′ and -6′); *m/e* (*cf.* ref. 18) 396 (M^+ 4%), 355 (75), 354 (80), 270 (100), 241 (38), 213 (13), 153 (23), 152 (19), 124 (23), 123 (25), and 118 (28); $\bar{\nu}$(KBr) 1 780, 1 655, and 1 210 cm^{-1}.

Apigenin 4′,7-dimethyl ether (14) obtained from (12) with diazomethane in ether–methanol and crystallization from chloroform–methanol, had m.p. 170—173° (lit.,[20] 170—171°); δ 3.87 (s, 4′- and 7-OMe), 6.35 (d, *J* 2 Hz, H-3), 6.47 (d, *J* 2 Hz, H-8), 6.53 (s, H-3), 7.02 (d, *J* 9 Hz, H-3′ and -5′), and 7.83 (d, *J* 9 Hz, H-2′ and -6′); *m/e* (*cf.* ref. 21) 298 (M^+, 100%), 269 (16), 255 (10), 166 (12), 162 (3), 138 (10), 135 (17), 132 (17), and 95 (14); $\bar{\nu}$(KBr) 3 230, 1 670, 1 610, 1 505, and 845 cm^{-1}.

Examination of Benzene-soluble Fraction.—T.l.c. (silica gel G; benzene–ether) of the benzene-soluble fraction (200 mg) gave a zone (R_F 0.55), which on elution and crystallization yielded dammarenediol-II (4) (29.4 mg), m.p. 132—133°.

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