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STRUCTURES OF PULVINONE DERIVATIVES FROM *ASPERGILLUS TERREUS*

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Key Word Index—*Aspergillus terreus*; Hyphomycetes; mould metabolite; pulvinone derivatives.

Abstract—The structures were established of six pulvinone derivatives, together with 3-(*p*-hydroxyphenyl)-4-hydroxy-5-(*p*-hydroxybenzylidene)-2(5H)-furanone (dihydroxy pulvinone), which were all isolated from a culture of *Aspergillus terreus*. Dihydroxy pulvinone is the fundamental structure for the six new compounds, which have one more hydroxyl group and/or two 3,3-dimethylallyl or related groups substituted on the aryl nuclei.

INTRODUCTION

Previously, we reported [1] the isolation from *Aspergillus terreus* of seven new butenolides. The simplest, compound E (**5**) is a dihydroxy derivative of 3-phenyl-4-hydroxy-5-benzylidene-2(5H)-furone called "pulvinone" by Claisen [2]. Among the other six compounds, one is a trihydroxy pulvinone and the other five have aryl nuclei substituted with two 3,3-dimethylallyl or related groups. Edwards *et al.* have also studied pulvinone and related compounds, including a trihydroxy pulvinone, isolated from higher fungi [3–5], but their compounds have no C_5 unit side chain and the trihydroxy derivative differs from ours. We now report the structural elucidation of our compounds.

RESULTS

The isolation and notation of the metabolites has been given in a previous paper [1]. The NMR spectrum of compound A (Table 1) showed a singlet at 1·31 (12H) and two triplets at 1·80 (4H) and 2·80 (4H) respectively indicating that two C_5 units form cyclic ethers on the aryl ring as shown in structure (**1**). The signals for the aryl ring protons were also consistent with this structure. The NMR spectrum of compound B showed that one side chain forms a cyclic ether, and that the other is in the 3,3-dimethylallyl form. Although it was not certain which of the two aryl rings carries the dimethylallyl substituent, structure (**2**) was tentatively given to this compound, by analogy to compound D whose structure was firmly established as described below. On heating in acid, compound B was converted to compound A. The NMR spectrum of compound C showed that both side chains were in the cyclic ether form. The dimethyl derivative (**8**) obtained by methylation with CH_2N_2 gave a keto-acid (**9**) on mild treatment with alkali. In the NMR spectrum of (**9**), a new singlet due to CH_2 appeared at 3·22 ppm and irradiation at this region caused an increase in peak height of one singlet (1H) at 7·14 ppm due to an aromatic proton. This fact indicated that the CH_2 formed by the fission of the lactone ring must be attached to the aryl ring carrying the extra hydroxyl group, as shown in structure (**9**). The existence of a *m*-dihydroxyphenyl group in naturally occurring aromatic compounds is rare except in the case of substances derived biogenetically from polyketide. For the further elucidation of the structure of compound C, its alkaline degradation was carried out, and the benzoic acid (**10**), phenylacetic acid (**11**), and its lactone (**12**) derivatives were obtained. Therefore, compound C has the structure (**3**).

The NMR spectrum of compound D showed clearly that the side chains were the same as those of compound B. On heating in acid, this was converted to compound C, and methylation with CH_2N_2 gave a trimethyl derivative. The fact that the methoxybenzoic acid derivative (**13**) was obtained as one of the degradation products of this

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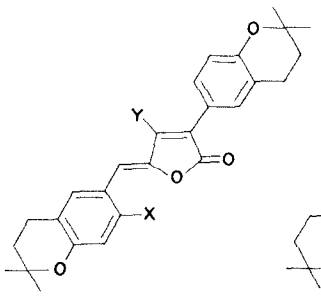
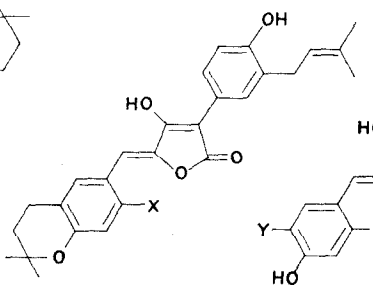
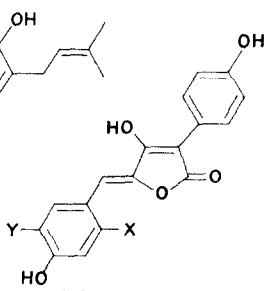


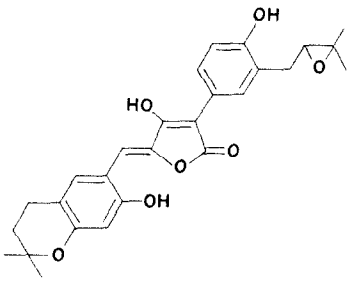
(1) X = H; Y = OH
(3) X = OH; Y = OH
(8) X = OMe; Y = OMe



(2) X = H
(4) X = OH



(5) X = H; Y = H
(7) X = OH; Y = H
(16) X = H; Y = OH



(6)

Table 1. NMR data of pulvinone derivatives

Compound	Proton chemical shifts (in ppm from TMS in DMSO)	
A. Dicyclic isomer of bis-(dimethylallyl) derivative of dihydroxypluvinone	7·75–7·45 (*m*, 4H), 6·80 (*d*, 1H), 6·78 (*d*, 1H), 6·60 (*s*, 1H),	2·80 (*t*, 4H), 1·80 (*t*, 4H) 1·31 (*s*, 12H)
B. Monocyclic isomer of bis-(dimethylallyl) derivative of dihydroxypluvinone	7·65–7·40 (*m*, 4H), 6·78 (*d*, 1H), 5·34 (*t*, 1H), 3·32 (*d*, 1H), 6·71 (*d*, 1H), 6·35 (*s*, 1H),	2·80 (*t*, 2H), 1·80 (*t*, 2H) 1·75 (*s*, 6H), 1·30 (*s*, 6H)
C. Dicyclic isomer of bis-(dimethylallyl) derivative of trihydroxypluvinone	7·55–7·40 (*m*, 2H), 7·16 (*s*, 1H), 6·76 (*d*, 1H), 6·28 (*s*, 2H),	2·74 (*t*, 4H), 1·78 (*t*, 4H) 1·29 (*s*, 6H), 1·27 (*s*, 6H)
D. Monocyclic isomer of bis-(dimethylallyl) derivative of trihydroxypluvinone	7·60–7·40 (*m*, 2H), 7·16 (*s*, 1H), 5·30 (*t*, 1H), 3·30 (*d*, 2H), 6·86 (*d*, 1H), 6·28 (*s*, 2H),	2·70 (*t*, 2H), 1·80 (*t*, 2H) 1·72 (*s*, 6H), 1·28 (*s*, 6H)
E. Dihydroxypluvinone	7·92 (*d*, 2H), 7·72 (*d*, 2H), 6·95 (*d*, 2H), 6·92 (*d*, 2H), 6·68 (*s*, 1H),	
F. Epoxide of D	7·62 (*d*, 1H), 7·48 (*dd*, 1H), 7·15 (*s*, 1H), 4·58 (*t*, 1H), 6·79 (*d*, 1H), 6·30 (*s*, 1H), 6·24 (*s*, 1H), 3·20 (*d*, 2H),	2·70 (*t*, 2H), 1·75 (*t*, 2H) 1·27 (*s*, 6H), 1·16 (*s*, 6H)
G. Trihydroxypluvinone	7·66 (*d*, 2H), 7·26 (*d*, 1H), 6·88 (*d*, 2H), 6·42 (*s*, 1H), 6·35 (*dd*, 1H), 6·34 (*d*, 1H),	

Figures in parentheses show the number of proton. Abbreviations: *s*—singlet; *d*—doublet; *dd*—double doublet; *t*—triplet; *m*—multiplet.

trimethyl derivative supported (**4**) as the structure of compound D.

Compound F contained one more oxygen atom than compound D and the NMR spectrum indicated that it may form an epoxide ring. Acid treatment of compound F gave compound (**14**) which had a benzofuran moiety. The alkaline degradation of (**14**) gave 5-carboxymethyl-2-*iso*-propyl benzofuran (**15**) indicating that compound F has structure (**6**). The analytical and NMR data and the methylation with CH_2N_2 indicated that compound G was a trihydroxy pulvinone derivative. By analogy to compounds C, D and F and comparison of its properties with those of the trihydroxy pulvinone (**16**) in the literature [5], it is concluded that compound G has structure (**7**).

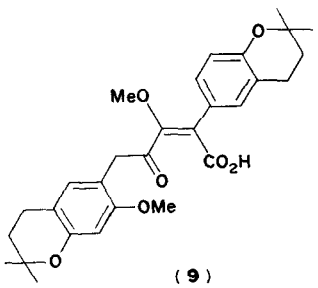
EXPERIMENTAL

All the metabolites were isolated from the culture of *Aspergillus terreus* IAM 2054 according to the procedure described before [1].

Compound A (**1**). M.p. 243–245° (Found: C, 74·51; H, 6·64. $C_{27}H_{28}O_5$ requires: C, 74·98; H, 6·53%); MS: 432 (M^+); UV: λ_{max}^{MeOH} 243 and 371 nm (log ε 4·34 and 4·49); IR: ν_{max}^{KBr} 1690, 1608, 1497 cm^{-1}.

Methylation of compound A. Compound A (70 mg) was treated with CH_2N_2 in Et_2O to give the monomethyl derivative of A which was recrystallized from MeOH (60 mg). M.p. 154–157°, (Found: C, 75·33; H, 7·02. $C_{28}H_{30}O_5$ requires: C, 75·31; H, 6·77%); UV: λ_{max}^{MeOH} 237 and 363 nm (log ε 4·31 and 4·54); IR: ν_{max}^{KBr} 1750, 1600, 1490 cm^{-1}; NMR ($CDCl_3$): δ 7·64 (*d*, 1H), 7·55 (*dd*, 1H), 7·3 (*dd*, 1H), 6·86 (*d*, 1H) 6·83 (*d*, 1H), 6·22 (*s*, 1H), 3·88 (*s*, 3H, C<u>H</u>$_3$O–), 2·82 (*t*, 4H), 1·82 (*t*, 4H), 1·34 (*s*, 12H).

Compound B (**2**). M.p. 187–189°, (Found: C, 72·52; H, 6·40. $C_{27}H_{28}O_5 . H_2O$ requires: C, 71·98; H, 6·71%); MS: 432 (M^+); UV: λ_{max}^{MeOH} 243 and 374 nm (log ε 4·33 and 4·49); IR: ν_{max}^{KBr} 1728, 1601, 1500 cm^{-1}.



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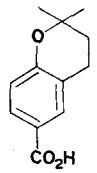

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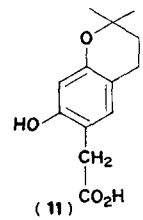

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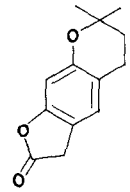

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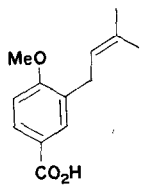

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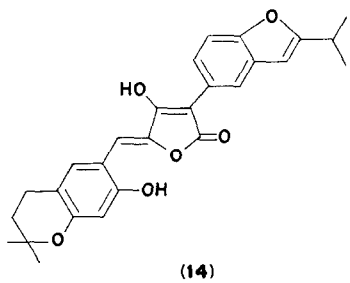

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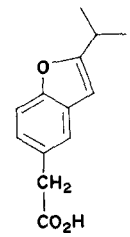

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Methylation of compound B. The dimethyl derivative of compound B was prepared as described above. M.p. 110–112°. (Found: C, 75·63; H, 7·04. $C_{29}H_{32}O_5$ requires: C, 75·63; H, 7·00%); UV: λ_{max}^{MeOH} 240 and 360 nm (log ε 4·37 and 4·60); IR: ν_{max}^{KBr} 1759, 1601, 1495 cm^{-1}; NMR ($CDCl_3$): δ 7·70 (*dd*, 1H), 7·47 (*d*, 1H), 7·27 (*d*, 1H), 7·22 (*dd*, 1H), 6·82 (*d*, 1H), 6·78 (*d*, 1H), 6·20 (*s*, 1H), 5·30 (*t*, 1H), 3·85 (*s*, 6H, $C\underline{H}_3O$–), 3·32 (*d*, 2H), 2·80 (*t*, 2H), 1·80 (*t*, 2H), 1·75 (*s*, 6H), 1·34 (*s*, 6H).

Compound C (**3**). M.p. 257–259°. (Found: C, 72·24; H, 6·23. $C_{27}H_{28}O_6$ requires: C, 72·30; H, 6·29%); MS: 448(M^+); UV: λ_{max}^{MeOH} 244 and 375 nm (log ε 4·23 and 4·50); IR: ν_{max}^{KBr} 1710, 1603, 1496 cm^{-1}.

Methylation of compound C. The dimethyl derivative (**8**) of compound C was prepared as described above. M.p. 157–162°. (Found: C, 72·79; H, 6·80. $C_{29}H_{32}O_6$ requires: C, 73·09; H, 6·77%); UV: λ_{max}^{MeOH} 238 and 356 nm (log ε 4·38 and 4·74); IR: ν_{max}^{KBr} 1760, 1610, 1492 cm^{-1}, NMR ($CDCl_3$): δ 7·55 (*d*, 1H), 7·47 (*dd*, 1H), 6·99 (*s*, 1H), 6·77 (*d*, 1H), 6·37 (*s*, 1H), 6·16 (*s*, 1H), 3·77 (*s*, 6H, $C\underline{H}_3O$–), 2·74 (*t*, 4H), 1·78 (*t*, 4H), 1·34 (*s*, 12H).

Compound D (**4**). M.p. 257–258°. (Found: C, 70·33; H, 6·65. $C_{27}H_{28}O_6.H_2O$ requires: C, 69·51; H, 6·48%); MS: 448(M^+); UV: λ_{max}^{MeOH} 245 and 377 nm (log ε 4·28 and 4·47); IR: ν_{max}^{KBr} 1720, 1600 cm^{-1}.

Trimethyl derivative of compound D. M.p. 88–90°. (Found: C, 73·18; H, 6·92. $C_{30}H_{34}O_6$ requires: C, 73·45; H, 6·99%); UV: λ_{max}^{MeOH} 238 and 351 nm (log ε 4·28 and 4·52); IR: ν_{max}^{KBr} 1756, 1607, 1497 cm^{-1}; NMR ($CDCl_3$): δ 7·83 (*dd*, 1H), 7·57 (*d*, 1H), 7·07 (*s*, 1H), 6·92 (*d*, 1H), 6·44 (*s*, 1H), 6·25 (*s*, 1H), 5·35 (*t*, 1H), 3·91 (*s*, 3H) and 3·82 (*s*, 6H, $C\underline{H}_3O$–), 3·36 (*d*, 2H), 2·78 (*t*, 2H), 1·80 (*t*, 2H), 1·76 (*s*, 6H), 1·35 (*s*, 6H).

Compound E. (**5**). M.p. 282–284°. (Found: C, 68·98; H, 4·04. $C_{17}H_{12}O_5$ requires: C, 68·91; H, 4·08%); MS: 296(M^+); UV: λ_{max}^{MeOH} 238 and 369 nm (log ε 4·32 and 4·46); IR: ν_{max}^{KBr} 1691, 1600, 1510 cm^{-1}.

Trimethyl derivative of compound E. M.p. 137–139°. (Found: C, 71·23; H, 5·03. $C_{20}H_{18}O_5$ requires: C, 70·99; H, 5·36%); UV: λ_{max}^{MeOH} 237 and 353 nm (log ε 4·26 and 4·52); IR: ν_{max}^{KBr} 1756, 1598, 1508 cm^{-1}; NMR ($CDCl_3$): δ 7·87 (*d*, 2H), 7·60 (*d*, 2H), 7·05 (*d*, 2H), 7·02 (*d*, 2H), 6·33 (*s*, 1H), 3·90 (*s*, 9H, $C\underline{H}_3O$–).

Compound F. (**6**). M.p. 234–235°. (Found: C, 70·07; H, 6·14. $C_{27}H_{28}O_7$ requires: C, 69·81; H, 6·08%); MS: 464(M^+); UV: λ_{max}^{MeOH} 242 and 375 nm (log ε 4·31 and 4·49); IR: ν_{max}^{KBr} 1720, 1605, 1490 cm^{-1}.

Dimethyl derivative of compound F. M.p. 118–125°. (Found: C, 70·45; H, 7·05. $C_{29}H_{32}O_7$ requires: C, 70·71; H, 6·55%); UV: λ_{max}^{MeOH} 238 and 357 nm (log ε 4·26 and 4·51); IR: ν_{max}^{KBr} 1760, 1610, 1491 cm^{-1}; NMR ($CDCl_3$): δ 7·72 (*d*, 1H), 7·41 (*dd*, 1H), 6·97 (*s*, 1H), 6·73 (*dd*, 1H), 6·35 (*s*, 1H), 6·16 (*s*, 1H), 4·60 (*t*, 1 H), 3·75 (*s*, 6H, $C\underline{H}_3O$–), 3·15 (*d*, 2H), 2·70 (*t*, 2H), 1·75 (*t*, 2H), 1·34 (*s*, 9H), 1·22 (*s*, 3H).

Compound G. (**7**). M.p. 263–265°. (Found: C, 64·76; H, 3·85. $C_{17}H_{12}O_6$ requires: C, 65·38; H, 3·87%), MS: 312(M^+); UV: λ_{max}^{MeOH} 237 and 369 nm (log ε 4·22 and 4·38); IR: ν_{max}^{KBr} 1737, 1608, 1513 cm^{-1}.

Tetramethyl derivative of compound G. M.p. 150–156°. (Found: C, 68·73; H, 5·46. $C_{21}H_{20}O_6$ requires: C, 68·47; H, 5·47%); UV: λ_{max}^{MeOH} 233 and 347 nm (log ε 4·31 and 4·54), IR: ν_{max}^{KBr} 1760, 1600, 1512 cm^{-1}; NMR ($CDCl_3$): δ 7·77 (*d*. 2H), 7·24 (*d*, 1H), 6·92 (*d*, 2H), 6·57 (*dd*, 1H), 6·50 (*d*, 1H), 6·24 (*s*, 1H), 3·85–3·80 (*m*, 12H, $C\underline{H}_3O$–).

Conversion of compound B to compound A. Compound B (10 mg) in MeOH (1 ml) and 6 N HCl (1 drop) was heated (70°) for 15 min. After evaporation of MeOH, the mixture was extracted with Et_2O to give a compound A in a quantitative yield.

Cleavage of the lactone ring of (**8**). To a solution of the dimethyl derivative of C (800 mg) in a mixture of Et_2O (40 ml) and MeOH (160 ml) were added 10% NaOH (8 ml) and H_2O (60 ml). The mixture was warmed at 45° for 72 hr. Evaporation of the organic solvents from the mixture under a reduced pressure followed by acidification with HOAc gave a white residue, which was recrystallized from MeOH, (**9**) (570 mg), m.p. 194–196°. (Found: C, 70·17; H, 7·09. $C_{29}H_{34}O_7$ requires: C, 70·42; H, 6·93%); UV: λ_{max}^{MeOH} 288 nm (log ε 3·82); IR: ν_{max}^{KBr} 1738, 1659 cm^{-1}; NMR ($CDCl_3$): δ 7·14 (*s*, 1H), 7·08 (*dd*, 1H), 6·80 (*d*, 1H), 6·56 (*d*, 1H), 6·36 (*s*, 1H), 3·72 (*s*, 3H), 3·68 (*s*, 3H), 3·22 (*d*, 2H), 2·71 (*m*, 4H), 1·76 (*m*, 4H), 1·32 (*s*, 12H).

Treatment of compound C with NaOH. Compound C (200 mg) with 6 N NaOH (15 ml) and EtOH (5 ml) was warmed at 80° for 40 hr under N_2. After cooling, the mixture was acidified with conc HCl and extracted with Et_2O to afford a colorless oil. This was chromatographed on a column of silica gel with *n*-hexane–Et_2O (7 : 3) to give three acidic materials (**10**), (**11**) and (**12**).

(**10**) (10 mg), m.p. 173–175°. (Found: C, 70·05; H, 7·04. $C_{12}H_{14}O_3$ requires: C, 69·88; H, 6·84%); MS: 206 (M^+); UV: λ_{max}^{MeOH} 260 nm (log ε 4·22); IR: ν_{max}^{KBr} 1675, 1608 cm^{-1}, NMR ($CDCl_3$): δ 10·6 (*b*, 1H), 7·87 (*d*, 1H), 7·85 (*dd*, 1H), 6·80 (*d*, 1H), 2·83 (*t*, 2H), 1·83 (*t*, 2H), 1·35 (*s*, 6H).

(**11**) (31 mg), m.p. 111–113°. (Found: C, 66·27; H, 6·93. $C_{13}H_{16}O_4$ requires: C, 66·08; H, 6·83%); MS: 236(M^+); UV: λ_{max}^{MeOH} 286 nm (log ε 3·63); IR: ν_{max}^{KBr} 3340, 1700, 1620 cm^{-1}; NMR ($CDCl_3$): δ 8·10 (*b*, 2H), 6·76 (*s*, 1H), 6·29 (*s*, 1H), 3·54 (*s*, 2H), 2·65 (*t*, 2H), 1·73 (*t*, 2H), 1·28 (*s*, 6H).

(**12**) (11 mg), m.p. 141–142°. (Found: C, 71·10; H, 6·15. $C_{13}H_{14}O_3$ requires: C, 71·54; H, 6·47%); MS: 218(M^+); UV: λ_{max}^{MeOH} 288 nm (log ε 3·60); IR: ν_{max}^{KBr} 1810, 1630 cm^{-1}; NMR (CCl_4): δ 6·86 (*s*, 1H), 6·46 (*s*, 1H), 3·50 (*s*, 2H), 2·73 (*t*, 2H), 1·77 (*t*, 2H), 1·30 (*s*, 6H).

Conversion of compound D to compound C. Compound D (1·0 g) in EtOH (20 ml) and conc HCl (10 ml) was warmed at 70° for 30 min. Pale yellow crystals which appeared on cooling, were collected and recrystallized (800 mg) from MeOH. It was identified as compound C by IR.

Treatment of trimethyl derivative of compound D with NaOH. A soln of the trimethyl derivative of compound D (200 mg) in MeOH (7 ml) and 6 N NaOH (10 ml) was stirred for 10 hr at reduced pressure and the residue acidified with conc HCl. The at 70° overnight. The organic solvents were removed under a reduced pressure and the residue acidified with conc. HCl. The mixture was then extracted with Et_2O, and the extract chromatographed on silica gel with *n*-hexane–ether (2:1) to give colorless crystals which were identical with the *p*-methoxy benzoic acid derivative (**13**) by IR (ν_{max}^{KBr} 1675, 1600 cm^{-1}), and MS (220, M^+).

Treatment of compound F with p-*toluenesulfonic acid.* A mixture of compound F (100 mg) and *p*-toluenesulfonic acid (300 mg) was heated at 115–120° for 10 min. After cooling, H_2O was added and the mixture extracted with Et_2O to give (**14**) (60 mg). It was purified by chromatography on silica gel with *n*-hexane–ether (2:1). M.p. 234–235°. (Found: C, 72·67; H, 5·68. $C_{27}H_{26}O_6$ requires: C, 72·63; H, 5·87%); UV: λ_{max}^{MeOH} 250, 314 and 368 nm (log ε 4·49, 4·36 and 4·37); IR: ν_{max}^{KBr} 1710, 1600 cm^{-1}; NMR [$(CD_3)_2SO$]: δ 8·07 (*d*, 1H), 7·78 (*dd*, 1H), 7·59 (*d*, 1H), 7·25 (*s*, 1H), 6·70 (*s*, 1H), 6·65 (*s*, 1H), 6·35 (*s*, 1H), 3·15 (*m*, 1H), 2·72 (*t*, 2H), 1·78 (*t*, 2H), 1·33 (*d*, 6H), 1·27 (*s*, 6H).

Dimethyl derivative of (**14**). M.p. 108–110°. (Found: C, 73·40; H, 6·61. $C_{29}H_{30}O_6$ requires: C, 73·40; H, 6·37%). UV: λ_{max}^{MeOH} 251 and 334 nm (log ε 4·42 and 4·49); IR: ν_{max}^{KBr} 1760, 1610 cm^{-1}; NMR ($CDCl_3$): δ 8·10 (*d*, 1H), 7·72 (*dd*, 1H), 7·46 (*d*, 1H), 7·07 (*s*, 1H), 6·44–6·40 (*s*, 3H), 3·82 (*s*, 3H) and 3·80 (*s*, 3H, CH_3O–), *ca* 3·12 (*m*, 1H), 2·78 (*t*, 2*H*), 1·82 (*t*, 2H), 1·36 (*d*, 6H), 1·33 (*s*, 6H).

Treatment of (**14**) *with* NaOH. (**14**) (200 mg) in 6 N NaOH (15 ml) and EtOH (5 ml) was refluxed for 20 hr under N_2. After evaporation of the EtOH, the mixture was acidified with conc HCl and extracted with Et_2O to afford a colorless oil which was chromatographed on silica gel. Elution with *n*-hexane–ether (4:1) gave two acidic materials (**11**) (35 mg) and (**15**) (11 mg). (**15**) m.p. 62–68°. (Found: C, 71·23; H, 6·53. $C_{13}H_{14}O_3$ requires: C, 71·54; H, 6·47%); MS: 218(M^+); UV: λ_{max}^{MeOH} 244, 280 and 286 nm (log ε 4·30, 3·76 and 3·80); IR: ν_{max}^{KBr} 1690, 1596 cm^{-1}; NMR (CCl_4): δ 10·9 (*b*, 1H), 7·41 (*d*, 1H), 7·38 (*d*, 1H), 7·12 (*dd*, 1H), 6·32 (*s*, 1H), 3·66 (*s*, 2H), *ca* 3·10 (*m*, 1H), 1·33 (*d*, 6H).

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