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ANTHOCYANIDINS AND RELATED COMPOUNDS—XII

TETRAMETHYLLEUCOCYANIDIN-PHLOROGLUCINOL AND RESORCINOL CONDENSATION PRODUCTS

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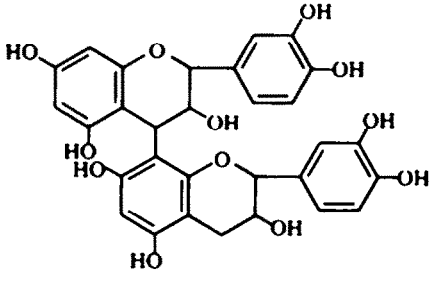
Abstract Crystalline phloroglucinol and resorcinol condensation products with 5,7,3',4'-tetramethoxyflavan-3,4-diol have been prepared. The structures of these products have been confirmed by extensive analytical and nuclear magnetic resonance spectral measurements.

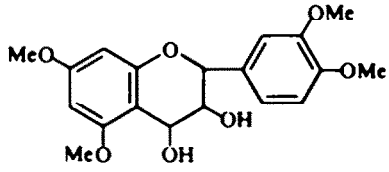
GEISSMAN and Dittmar,[1] Weinges and Freudenberg,[2] and Creasy and Swain[3] recently reported the isolation of natural, dimeric proanthocyanidins, in which flavan-3-ol nuclei are linked by an acid labile C to C bond from the 4-position of one to the 6- or 8-position at the phloroglucinol nucleus of the other, e.g. (I). Geissman and Dittmar[1] suggested that the dimers arise from a leucocyanidin–catechin condensation and in support of this type of C to C condensation Geissman and Yoshimura[4] have demonstrated that tetramethylleucocyanidin II condenses with phloroglucinol and with catechin under mild (0·1N HCl) acidic conditions[5] to give proanthocyanidin products. In the case of the phloroglucinol reaction they found that equal quantities of two major products were formed. The trimethylsilyl ether of one of these was obtained in a chromatographically homogeneous condition and its NMR spectrum was in accord with structure IIIa for the corresponding phenolic condensation product.

We wish to report that the tetra-O-methylleucocyanidin–phloroglucinol condensation product and its acetyl and methyl derivatives have now been crystallized. The properties of these pure compounds fully confirm the principal observations of Geissman *et al.*[1] Thus, with a large excess of phloroglucinol in dilute, aqueous acetic acid solutions or in aqueous buffer solutions (pH 3·2) each of the isomeric 5,7,3',4'-tetramethoxyflavan-3,4-diols (m.p. 207° and m.p. 163–164°) rapidly form the same, *single* crystalline condensation product, $C_{25}H_{26}O_9$, in yields of about 90%. With acetic anhydride and pyridine, this forms a highly crystalline tetraacetate, $C_{33}H_{34}O_{13}$. On methylation it yields a crystalline trimethyl derivative, $C_{28}H_{32}O_9$, which forms a monoacetate when acetylated. The condensation product, therefore, contains 4 free OH groups, three of which are phenolic and one of which is alcoholic. An ether linkage between the flavan and phloroglucinol nuclei is thereby excluded. Heated with dilute, aqueous acetic acid it is partially hydrolyzed to phloroglucinol. Heated with n-butanol–6N HCl[6] the condensation product gave tetra-O-methylcyanidin chloride (λ_{max} 533 mμ), the amount of anthocyanidin formed being almost identical with that obtained from an equimolecular quantity of the flavan-3,4-diol under the same conditions. These data are in complete agreement with structure IIIa for the

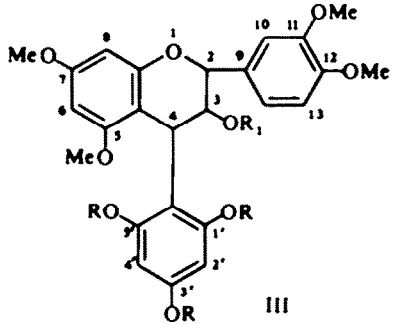
condensation product. The alcoholic OH of IIIa is hindered. Heated with acetic anhydride and potassium carbonate in acetone IIIa forms a crystalline triacetate, which may be converted into the tetraacetate IIIb with acetic anhydride and pyridine. Acetylation of d-catechin under these conditions yields its pentaacetate. The triacetate does not appear to contain a phenolic hydroxyl group and is considered to be IIIe, a conclusion supported by its NMR spectrum (*vide infra*).



I



II



III

IIIa: R = R_1 = H
IIIb: R = R_1 = Ac
IIIc: R = Me, R_1 = H
IIId: R = Me, R_1 = Ac
IIIe: R = Ac, R_1 = H

As in the case of phloroglucinol, resorcinol readily condenses with II to yield the crystalline proanthocyanidin IVa. In this and in the phloroglucinol reaction the formation of a second product was not detected. It would seem probable that the second product observed by Geissman and Yoshimura[4] arises from the further condensation of the phloroglucinol nucleus of IIIa with the diol.

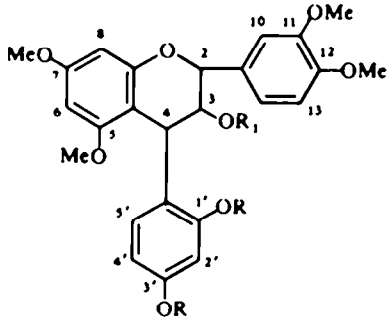
PMR spectra of several derivatives of III and IV provide unambiguous evidence for the structure of these compounds. Table 1 gives the shielding values for II (isomer, m.p. 163–164°), several derivatives of III, and one derivative of IV. Previously published NMR data on flavan-3,4-diols[7–10] together with the areas and splitting patterns of the observed multiplets and the selective shifts caused by acetylation made most of the assignments quite straightforward. Other possible structures such as Va and Vb in which the hydroxyl carbon is adjacent to only a single hydrogen-bearing carbon can be ruled out by the triplet splitting of the carbinol proton resonance. The large shift of this multiplet to lower field (1·54–1·55 ppm) upon acetylation provides a positive means for its assignment. This shift is unusually large and is perhaps further evidence for the 3,4-disubstituted structure since Clark-Lewis *et al.*[10] report similarly large shifts for 3,4-diols (1·5–1·7 ppm) while finding normal values (1·2 ppm) for several 3-ols.

TABLE 1. PROTON SHIELDING VALUES FOR TETRAMETHYLLEUCOCYANIDIN AND DERIVATIVES OF ITS CONDENSATION PRODUCTS

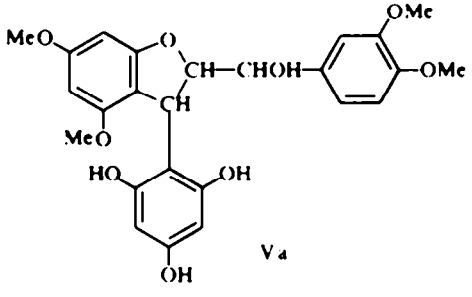
Compound	Shielding values (δ)* Ring H								MeO				AcO	
	2,4	3	6,8	10	13	14	2',4'	5'	7,11,12	1',5'	5	3'	1',3',5'	3
II	5·02 4·90	3·97	6·10	7·00	6·89	7·04			3·89 3·88 3·72		3·85			
III-b	4·78 4·60	5·79	6·01 6·15	6·98	6·85	7·03	6·88 6·77		3·91 3·88 3·72		3·38		2·35 2·22 1·94	1·67
III-c	4·63 4·58	4·26	5·99 6·15	7·04	6·88	7·07	6·12 (br)		3·90 3·88 3·72	3·42 (br) 3·88 (br)	3·38	3·78		
III-c – 50°	4·65	4·34	5·99 6·15	7·02	6·91	7·11	6·06 6·20		3·91 3·91 3·74	3·91 3·47	3·41	3·81		
III-d	4·73 (sl br)	5·78	5·99 6·16	6·99	6·82	7·04	6·06		3·88 3·85 3·72	3·67 (br)	3·36	3·74		1·66
III-d – 50°	4·72 4·77	5·85	6·02 6·17	6·98	6·87	7·09	6·05 6·13		3·92 3·88 3·75	3·94 3·55	3·42	3·79		1·70
III-e	4·56 4·42	4·24	5·99 6·13	6·99	6·88	7·04	6·85 6·71		3·90 3·88 3·71		3·39		2·34 2·22 1·92	
IV-b	4·66 4·50	4·02	6·07 6·21	6·96	6·84	7·02	6·47 6·32	6·66	3·85 3·85 3·75	3·84	3·41	3·75		

* Ppm from internal TMS measured at 100M Hz in $COCl_3$, at 30° unless otherwise specified.

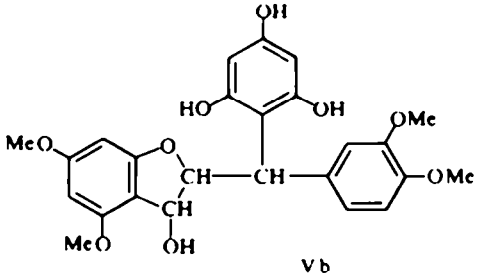
The magnitude of the two vicinal proton couplings of the C-ring provide a direct means for the assignment of its conformation. In the spectrum of II in deuterochloroform the 3-proton absorption is partially obscured by the lowest field methoxyl resonance. However, in pyridine-d_5 the absorption of the three C-ring hydrogens can be observed, and they are sufficiently separated to allow the use of first-order analysis, which yielded couplings to the 3-proton of 10·2 Hz and 3·8 Hz. The former value is characteristic of a trans diaxial coupling while the latter corresponds to a gauche interaction. Since one of the couplings to the 3-proton is diaxial, this proton must be axial. Of the two remaining hydrogens, one is axial, the other equatorial. In order to assign the 2-proton and 4-proton absorptions unambiguously, trichloroacetyl isocyanate, which adds stoichiometrically to OH groups causing a major downfield shift of the carbinol proton[9] somewhat larger than that produced by acetylation, was added to the pyridine solution. The doublet-split doublet (10·2,



IVa: R = R_1 = H
IVb: R = Me, R_1 = H
IVc: R = Me, R_1 = Ac



Va



Vb

3·8 Hz), and the 3·8 Hz doublet shifted 1·4 ppm downfield, while the position of the 10·2 Hz doublet shifted 0·5 ppm upfield. Thus, the 4-proton is equatorial and the 2-proton is axial in II.

In the spectrum of IIIb, acetylation of the 3-OH group shifts the 3-proton resonance sufficiently downfield in respect to the distinct 2- and 4-proton absorptions so that a first-order analysis can be applied, yielding di-axial couplings to the 3-proton of 9·2 Hz and 9·8 Hz. Thus, in the course of condensation inversion of the 4-proton occurs. A similar inversion has been reported by Fujisë *et al.*[11] for the 4a-proton in flavan-3,4-diols during the formation of isopropylidene derivatives, and Dreues and Roux[12] have proposed a possible mechanism based on electron-release from the 7-methoxyl which is reinforced by the presence of a 5-OMe, as in II. Evidence from 100M Hz NMR of similar inversion during the course of acetylation of a flavan-4β-ol has been discussed by Krishnamurty *et al.*[13]

The spectra of IIIc and IIId, in which the phloroglucinol hydroxyls have been methylated, display several distinctive spectral features. Spectra of IIIc at −50°, 30°, and 50° are shown in Fig. 1. The area of the OMe region at room temperature shows the presence of 7 Me groups. Five of these give rise to sharp peaks, the remaining two produce a single broad peak in the case of IIId and two broad peaks in the case of IIIc. Additionally, in the case of IIIc the 2'- and 4'-proton absorptions consist of a

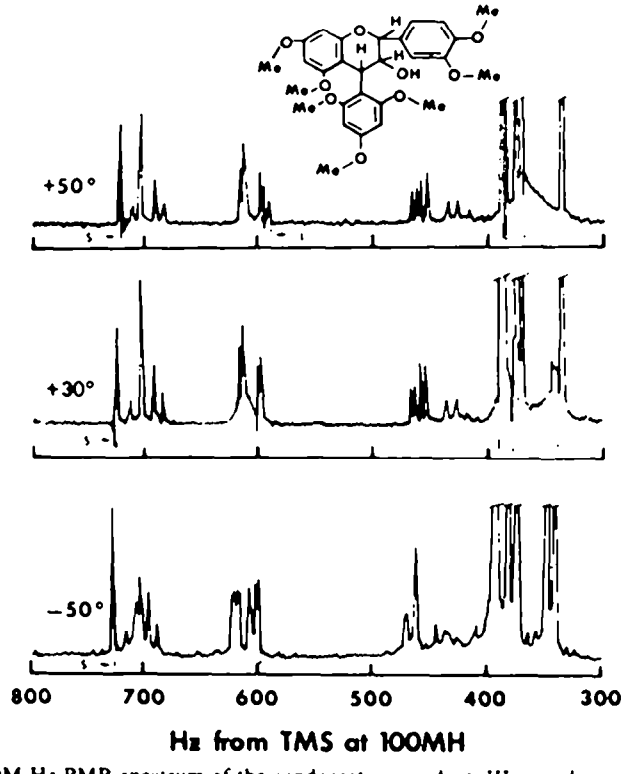


FIG. 1 100M Hz PMR spectrum of the condensation product, IIIc, in deuterochloroform at −50°, 30°, and 50°.

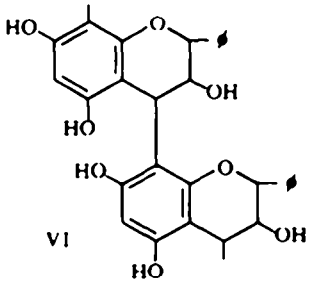
single broad peak having a width of 15 Hz. In the case of IIId, the peak representing these protons is only slightly broadened (width about 2 Hz), but the base of the doublet representing the 2- and 4-protons shows considerable broadening.

At 50° all of the broadened peaks are appreciably sharper singlets. In the case of IIIc, the two broad methoxyl resonances coalesce into a single broad peak. With tetrachloroethane as solvent this peak becomes sharper even at 90°.

At −50° all selective broadening vanishes. In the case of both IIIc and IIId, one OMe is situated at the low field end of the aromatic OMe range at about 3·9 ppm while the other is found 0·4 ppm to higher field. The singlet assigned to the 2'- and 4'-protons at higher temperatures splits into two doublets with a typical *meta* coupling of 2 Hz.

Such temperature behavior is characteristic of a system containing a hindered rotor. In this case, the hindered group must be the phloroglucinol ring when substituted at the 2' and 4' positions by O-Me groups. When one of these groups is missing, as in IVb, this behavior is not observed at room temperature. On the other hand, the substitution of O-acetyl groups at the 2' and 4' positions essentially freezes the ring in one conformation at room temperature as shown by the non-equivalence of the three acetyl groups.

As previously mentioned, equimolecular quantities of the condensation product IIIa and of 5,7,3',4'-tetramethoxyflavan-3,4-diol form approximately identical quantities of 5,7,3',4'-tetra-O-methylcyanidin chloride with butanolic–HCl. Slight changes in the experimental conditions result in highly variable yields of the anthocyanidin. In one experiment, however, IIIa, the diol (m.p. 163°) and the diol (m.p. 207°) gave 29·6%, 29·6% and 30·0%, respectively, of the theoretical amount of the flavylium salt, when heated simultaneously under the same conditions with butanolic-HCl. These figures are in fair agreement with those of Creasy and Swain,[3] who found that the avocado and strawberry dimers gave 35–38% or the theoretical amount of cyanidin chloride. These data indicate that polymers containing C—C bonds of the type present in the phloroglucinol and catechin condensation products should also give anthocyanidins in about the same yields (30%) as monomeric flavan-diols. It has been reported, however, that the yields of anthocyanidins from polymerized leucofisetinidin,[14] leucocyanidin[3] and leucodelphinidin[15] are only 5–17%. If these figures represent the maximum amounts of anthocyanidins obtainable under given conditions, its would seem that polymerization of flavan-3,4-diols, alone or with dimers of type I, to yield tannins must involve other structural variations in addition to the formation of repeating units of type VI.



VI

The isomeric 5,7,3',4'-tetramethoxyflavan-3,4'-diols used in this study were prepared by sodium borohydride reduction of 5,7,3',4'-tetra-O-methyl dihydroquercetin. This reaction was previously described by Swain[16] and Robertson[17] who obtained products melting at 123–125° and 175–190°, respectively. Ganguly and Seshadri[18] obtained two diols, m.p. 172° and 198°, which were separated by fractional crystallization. In agreement with Fujisë *et al.*,[19] we obtained crystalline isomeric diols, m.p. 207° and 163–164°, readily separated by virtue of the formation of a water-soluble borate complex by the lower melting isomer.

EXPERIMENTAL

5,7,3',4'-Tetramethoxyflavan-3,4-diol II

A suspension of 5,7,3',4'-tetra-O-methyldihydroquercetin (10·0 g)[20] in MeOH (250 ml) at 50° was treated during 10 min with $NaBH_4$ (10·0 g). Colorless crystals began to separate from the clear soln. After 1 hr, water (100 ml) was added and the crystalline product (m.p. 205–206°) was collected (3·1 g). Recrystallized from a large volume of acetone–MeOH pure 5,7,3',4'-tetramethoxyflavan-3,4-diol separated as colorless prisms, m.p. 207°, λ_{max}^{EtOH} 275 (3·50), 228 (4·24) mμ (log ε). (Found: C, 63·1; H, 6·05; MeO—, 34·6. Calc. for $C_{19}H_{22}O_7$: C, 63·0; H, 6·12; 4 MeO, 34·3%).

The aqueous MeOH reaction filtrate from the above diol was diluted with water (500 ml) and the clear soln was acidified with glacial AcOH (40 ml). The isomeric diol rapidly separated as a gelatinous mass. This was recrystallized twice from MeOH. 5,7,3',4'-tetramethoxyflavan-3,4-diol was thus obtained as colorless, felted needles, m.p. 163–164° (4·90 g), λ_{max}^{EtOH} 275 (3·49), 228 (4·23) mμ (log ε). (Found: C, 63·0; H, 6·08; MeO—, 34·6. Calc. for $C_{19}H_{22}O_7$: C, 63·0; H, 6·12; 4 MeO—, 34·3%).

Condensation of 5,7,3',4'-tetramethoxyflavan-3,4-diol (m.p. 207°) with phloroglucinol

(a) A mixture of the diol (2·0 g) and phloroglucinol (10·0 g) was heated briefly to boiling. The clear soln was diluted with warm water (500 ml) and allowed to stand overnight at room temp. IIIa crystallized as small, colorless needles, m.p. 257° (2·27 g; 87·3% theory).

(b) A soln of the diol (1·0 g) and phloroglucinol (5·0 g) in AcOH (50 ml) and water (250 ml) was treated with 10% HCl aq (10·0 ml) at 50°. IIIa separated as colorless needles, m.p. 257° (0·75 g).

Recrystallized from acetone–MeOH IIIa was obtained as colorless needles, m.p. 257–258°, λ_{max}^{EtOH} 278 (3·69), λ_{max}^{NaOEt} 358, shoulders at 284, 279 mμ (log ε). IIIa gave an orange-red color with acidified ethanolic vanillin solns and on paper chromatograms it migrates as a single substance (R_f 0·88 in 50% aqueous AcOH. Phloroglucinol, as a reference, had R_f 0·71 in this solvent). (Found: C, 63·3; H, 5·62; MeO—, 26·2. Calc. for $C_{23}H_{26}O_9$: C, 63·8; H, 5·57; 4 MeO, 26·4%).

IIIa (0·20 g) was warmed with Ac_2O (1·0 ml) and pyridine (5 drops) for 5 min. The acetate IIIb obtained on adding water crystallized from MeOH as glistening, colorless needles, m.p. 188–189°, λ_{max}^{EtOH} 276 mμ. IIIb migrated as a single substance on silicic acid TLC. (Found: C, 62·2; H, 5·39; MeO—, 19·4; Ac—, 26·8. Calc. for $C_{33}H_{34}O_{13}$: C, 62·0; H, 5·37; 4 MeO—, 19·4; Ac—, 26·9). A mixture of IIIa (0·20 g), K_2CO_3 (6·0 g), Ac_2O (2·0 ml) and anhyd acetone (20·0 ml) was heated under reflux for 30 min and filtered. The filtrate was evaporated to an oil and treated with water. The solid acetate thus obtained was recrystallized from a mixture of acetone and MeOH. The *triacetate* IIIc separated as glistening, colorless prisms, m.p. 202° (0·15 g). (Found: C, 62·5; H, 5·33; MeO—, 20·9, Ac—, 21·3. Calc. for $C_{31}H_{32}O_{12}$: C, 62·4; H, 5·41; 4 MeO—, 20·8; 3 Ac, 21·6%).

The triacetate IIIc was acetylated with warm Ac_2O and pyridine. The tetraacetate IIIb was obtained, m.p. and m.m.p. 188–189°.

IIIa (0·50 g) was heated under reflux with Me_2SO_4 (5·0 ml), K_2CO_3 (10·0 g) and acetone (50 ml). The mixture was concentrated and diluted with water. After 1 hr the solid product was collected and recrystallized from acetone–MeOH. The *trimethyl derivative* IIIc was obtained as colorless prisms, m.p. 200° (0·48 g). (Found: C, 65·5; H, 6·22; MeO, 42·3. Calc. for $C_{28}H_{32}O_9$: C, 65·6; H, 6·29; 7 MeO, 42·4%).

Warmed with Ac_2O and pyridine the trimethyl derivative formed a *monoacetate* IIId. This crystallized from MeOH as glistening, colorless needles, m.p. 162–163°. (Found: C, 65·1; H, 6·20; MeO—, 39·2; Ac, 7·43. Calc. for $C_{30}H_{34}O_{10}$: C, 65·0; H, 6·18; 7 MeO—, 39·2; 1 Ac, 7·76%).

The tetraacetate IIIb (0·15) was heated under reflux with Me_2SO_4 (2·0 ml), K_2CO_3 (5·0 g), acetone

(15·0 ml) and MeOH (5·0 ml) for 2 hr. The mixture was concentrated and diluted with water. The product IIId crystallized from MeOH as colorless needles, m.p. and m.m.p. 162–163°.

Condensation of 5,7,3',4'-tetramethoxyflavan-3,4-diol (m.p. 163–164°) with phloroglucinol

(a) The diol (1·90 g) was condensed with phloroglucinol (10·0 g) in aqueous AcOH as described above. IIIa crystallized as colorless needles, m.p. and m.m.p. 257–258°; tetraacetate, m.p. and m.m.p. 188–189° (2·05 g).

(b) The diol (0·1 g) and phloroglucinol (0·5 g) were dissolved in EtOH (5·0 ml). Citric acid–sodium phosphate buffer (pH 3·2) (25·0 ml) was slowly added. Within 10 min the soln became cloudy and IIIa began to precipitate. After 8 hr this was collected (0·95 g) and recrystallized, m.p. 257–258° (tetraacetate m.p. and m.m.p. 188–189°).

5,7,3',4'-Tetra-O-methylcyanidin chloride

5,7,3',4'-Tetra-O-methylcyanidin chloride has λ_{max} 532 mμ (log ε 4·54) in n-butanol–HCl.[21] (a) 5,7,3',4'-Tetramethoxyflavan-3,4-diol (m.p. 163–164°; 0·0046 g) was heated in a steam bath with n-butanol–12N HCl reagent[6] (10·0 ml) for 70 min and diluted to 250·0 ml with the reagent. The absorbance of the soln at λ_{max} 532 mμ was 0·521 (29·56% theoretical amount of the anthocyanidin). (b) The diol (m.p. 207°; 0·0030 g) under identical conditions gave a soln, λ_{max} 532 mμ, absorbance 0·345 (30·02% theoretical amount of anthocyandin. (c) IIIa (0·0057 g) under identical conditions gave a soln, λ_{max} 532 m.p., absorbance 0·497 (29·60% theoretical amount of anthocyanidin).

The pigments obtained in (a), (b), and (c) had identical R_f values in water–acetic acid–12N HCl, 80:40:5 (R_f 0·57) and 80:20·5 (R_f 0·37) and in formic acid–3N HCl, 1:1 (R_f 0·66).

Condensation of 5,7,3',4'-tetramethoxyflavan-3,4-diol with resorcinol

The diol (m.p. 207°; 0·30 g) was treated with resorcinol (3·0 g) in AcOH (15·0 ml) and water (75·0 ml) as described above. The colorless, crystalline condensation product was recrystallized from acetone–MeOH. IVa separated as colorless prisms, m.p. 246° (0·25 g). (Found: C, 66·1; H, 5·76. Calc. for $C_{25}H_{26}O_8$: C, 66·1; H, 5·77%).

Refluxed with Me_2SO_4 and K_2CO_3 in acetone the condensation product formed a dimethyl derivative IVb, which crystallized from acetone–MeOH as colorless, brittle prisms, m.p. 143–144°. (Found: C, 67·2; H, 6·20. Calc. for $C_{27}H_{30}O_8$: C, 67·2; H, 6·27%).

Acetylation of IVb with Ac_2O and pyridine gave the monoacetate IIIc, colorless prisms ex-MeOH, m.p. 145°. (Found: C, 66·5; H, 6·17. Calc. for $C_{29}H_{32}O_9$: C, 66·4, H, 6·15%).

PMR absorption and integral spectra were taken at 100M Hz on an internally locked Varian HR-100 spectrometer with a variable temp probe using $CDCl_3$ as the solvent and TMS as the locking signal. When temps above 50° were desired, 1,1,22-tetrachloroethane was used as a solvent, and pyridine-d_5 was used in one case to produce selective peak shifts.

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