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DILATATIN, THE FIRST EXAMPLE OF A *C*-ALLOSYLATED NATURAL PRODUCT

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Key Word Index—*Hymenophyllum dilatatum*; Hymenophyllaceae; Filicales; dilatatin; xanthone *C*-glycoside; allose; 2-*C*-β-D-allopyranosyl-1,3,6,7-tetrahydroxyxanthone; dilatatin; synthesis.

Abstract—Dilatatin, the xanthone-*C*-glycoside previously isolated from the filmy fern *Hymenophyllum dilatatum*, is identified as 2-*C*-β-D-allopyranosyl-1,3,6,7-tetrahydroxyxanthone. The glycosyl moiety is demonstrated to be allopyranose by ^{13}C NMR spectroscopy using synthetic β-D-allopyranosyl-2,4,6-trimethoxybenzene as a model.

INTRODUCTION

In 1980 Markham and Wallace [1] reported the isolation of a new *C*-glycosylxanthone from the filmy fern *Hymenophyllum dilatatum*. The aglycone moiety of this glycoside was shown unequivocally to be 1,3,6,7-tetrahydroxyxanthone and elemental analysis and acid treatment defined dilatatin as a mono-*C*-hexoside of this xanthone, containing no other substituents. On the basis of absorption data and chromatographic evidence dilatatin was initially thought to be mangiferin [2-*C*-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone (**1**)], a known constituent of many other leptosporangiate ferns [1, 2]. Closer study, however, revealed differences in the optical rotation and ^1H NMR and ^{13}C NMR spectra. The present communication extends this work and identifies dilatatin as 2-*C*-β-D-allopyranosyl-1,3,6,7-tetrahydroxyxanthone (**3**).

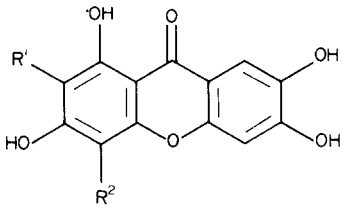
RESULTS AND DISCUSSION

The site of the sugar linkage to the aglycone in dilatatin was considered to be at C-2 of 1,3,6,7-tetrahydroxyxanthone, since in the ^{13}C NMR spectrum, the one protonated carbon (C-4) in the phloroglucinol ring appeared at δ 94 (cf. mangiferin, δ 93.7) and the glycosylated carbon (C-2) at δ 107.8 (cf. mangiferin, δ 107.7) [1]. Furthermore, dilatatin (**3**) bore the same chromatographic relationship to its Wessely–Moser rearrangement isomer, isodilatatin, as mangiferin did to isomangiferin (**2**).

The hexose substituent at C-2 gave a pattern of ^{13}C NMR signals markedly different from those exhibited by the *C*-linked glucose in mangiferin (Table 1) and from other *C*-linked hexoses for which ^{13}C NMR data were available, e.g. galactose and rhamnose [3]. It was possible, however, to calculate approximately the spectra of unknown *C*-hexosides from those of *O*-hexosides by using differences observed between the spectra of known *C*- and *O*-linked hexosides. Such calculations suggested allose to be the most likely sugar residue in dilatatin. This was supported also by ^{13}C NMR data on a comprehensive series of 1,5-anhydrohexitols.* Only the spectrum of 1,5-anhydroallitol approximated closely to that of the sugar in dilatatin when allowance was made for the effects on the chemical shifts brought about by bonding to a phloroglucinol ring system (cf. ref. [3]).

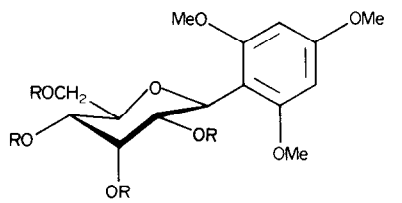
It was therefore decided to synthesize an authentic *C*-alloside for ^{13}C NMR comparison, and for convenience β-D-allopyranosyl-2,4,6-trimethoxybenzene (**4**) was chosen. β-Linkage of the sugar was indicated by the ^1H NMR spectrum of dilatatin in which the sugar H-1′ signal at δ 5.05 exhibited *trans*-diaxial coupling (J = 9.4 Hz) with the adjacent C-2 proton. The tetraacetate (**5**) of the chosen model was synthesized in 44% yield by reacting 2,3,4,6-tetraacetyl-α-D-allopyranosyl bromide

(A)



1 R^1 = β-D-glucopyranosyl; R^2 = H (mangiferin)
2 R^1 = H; R^2 = β-D-glucopyranosyl (isomangiferin)
3 R^1 = β-D-allopyranosyl; R^2 = H (dilatatin)

(B)



4 R = H
5 R = COMe

*Some available from ref. [3], others (1,5-anhydro-mannitol, -allitol, -talitol and altritol) kindly made available by Dr. V. M. Chari, formerly of the University of Munich.

Table 1. Chemical shifts of the sugar carbons in the ^{13}C NMR spectra of various *C*-glycosylated phenols

Compound	Sugar carbon resonances (δ)					
	C-1	C-2	C-3	C-4	C-5	C-6
Mangiferin (**1**)	73.4	70.6*	79.2	70.8*	81.7	61.7‖
Dilatatin (**3**)	71.9	68.9†	68.2†	68.1†	76.7	61.8‖
β-D-Allopyranosyl-2,4,6-trimethoxybenzene (**4**)	71.7	68.5‡	68.5‡	67.8‡	76.2	62.2‖
β-D-Glucopyranosyl-2,4,6-trimethoxybenzene	73.2	70.5§	79.2	70.9§	81.3	61.8‖

*, †, ‡, §Assignments bearing the same superscript may be reversed.
‖C–H coupling: triplet J = *ca* 140 Hz, all other signals doublets J = *ca* 140 Hz.

with tri-*O*-methylphloroglucinol in dry carbon tetrachloride in the presence of zinc oxide. The constitution of the product was confirmed by elemental analysis and ^1H NMR spectroscopy. Deacetylation of this product with sodium methoxide in dry methanol gave β-D-allopyranosyl-2,4,6-trimethoxybenzene (**4**) in 75% yield. ^{13}C NMR and ^1H NMR spectroscopy confirmed the identity of the product, the latter also confirming that the β-linkage of the allose had been produced (H-1′, *d*, J = 9.9 Hz at δ 4.93).

The ^{13}C NMR spectrum of **4** (Table 1) resembled very closely that of dilatatin in the sugar carbon region, and this evidence was taken to provide firm confirmation that the *C*-linked sugar in dilatatin was indeed β-D-allopyranose. That the nature of the aglycone in **4** had little effect on the chemical shifts of the sugar carbon was evident from a comparison (see Table 1) of the sugar carbon resonances in mangiferin and β-D-glucopyranosyl-2,4,6-trimethoxybenzene (also synthesized by the same route as **5**).

Allose is a sugar which is rare in nature. Only very recently has it been found in glycosidic combination with naturally occurring phenols [4, 5]. Dilatatin appears to be the first example of a natural product containing a *C*-linked β-D-allopyranosyl moiety.

EXPERIMENTAL

NMR measurements were made on a Varian FT-80A instrument at 20 MHz (^{13}C) and 80 MHz (^1H).

2,3,4,6-Tetraacetyl-α-D-allopyranosyl bromide. 2,3,4,6-Tetraacetyl-α-D-allopyranosyl bromide was prepared by dissolution of β-D-allose pentaacetate (**6**) [6] in an excess of a 33% soln of HBr in HOAc maintained at ambient temp. After dilution with $CHCl_3$ and washing with H_2O and then with saturated $NaHCO_3$ soln, the organic phase was dried and concd to a colourless, immobile gum which was characterized by the presence, in the ^1H NMR spectrum, of a clean one-proton doublet (H-1) centred at δ 6.50 (J = 7.5 Hz).

β-D-Allopyranosyl-2,4,6-trimethoxybenzene tetraacetate (**5**). From a suspension of dry ZnO (3.00 g) and Drierite (3.50 g) in dry CCl_4 (75 ml) containing trimethylphloroglucinol (1.03 g, 6.13 mmol) was distilled *ca* 30 ml solvent. To the remaining refluxing mixture was added, dropwise over 15 min, a soln of 2,3,4,6-tetraacetyl-α-D-allopyranosyl bromide [prepared from 0.60 g (1.45 mmol) β-D-allopyranose pentaacetate] in dry CCl_4 (10 ml). After refluxing for 2.5 hr, the mixture was filtered through Celite, the inorganic materials were washed with hot CCl_4 (2 × 50 ml), and the combined organic phases concd to a dark red oil (1.31 g) which slowly solidified. The total product was subjected to prep. TLC on silica gel and eluted with 40% EtOAc–toluene. Trimethylphloroglucinol (0.76 g) was recovered from the most mobile species, R_f *ca* 0.9. A band visible in UV light at R_f 0.6 was eluted with EtOAc and concd to an immobile gum (0.41 g, 57%) and purified by further chromatography on silica gel to give 0.34 g of β-D-allopyranosyl-2,4,6-trimethoxybenzene tetraacetate (**5**) as a colourless, immobile gum, $[\alpha]_D$ +9.8° (*c* 1.2 in $CHCl_3$) (Found: C, 55.3; H, 6.2. $C_{23}H_{30}O_{12}$ requires: C, 55.4; H, 6.1%). ^1H NMR ($CDCl_3$): δ 1.77 (*s*, 3H, OAc), 2.04 (*s*, 6H, OAc), 2.21 (*s*, 3H, OAc), 3.78 (*s*, 3H, OMe), 3.80 (*s*, 6H, OMe), 5.31 (*d*, 1H, J = 10.0 Hz, H-1 allose), 6.09 (*s*, 2H, aromatic). Signals attributable to the remaining allopyranosyl ring protons appeared as broad multiplets centred at δ 4.19 (3H), 5.10 (1H) and 5.80 (2H).

β-D-Allopyranosyl-2,4,6-trimethoxybenzene (**4**). A soln of **5** (0.40 g, 0.80 mmol) in dry MeOH (20 ml) containing a catalytic amount (*ca* 0.05 g) of Na was stirred for 0.5 hr at ambient temp. before being neutralized with ion exchange resin (Dowex 50W-X8, H). Concn of the supernatant organic phase furnished a colourless, immobile gum (0.23 g) which was purified by prep. TLC in Me_2CO–EtOAc–H_2O (5:4:1). The band at R_f 0.5 gave β-D-allopyranosyl-2,4,6-trimethoxybenzene (**4**) (0.20 g, 75%) as a colourless gum, $[\alpha]_D$ 1.1° (*c* 1.183 in MeOH); ^1H NMR (d_6-DMSO): δ 3.69 (*s*, 6H, OMe), 3.74 (*s*, 3H, OMe), 4.93 (*d*, 1H, J = 9.9 Hz, anomeric proton), 6.16 (*s*, 2H, aromatic). Signals attributable to the remaining allopyranosyl ring protons were not clearly recognizable but appeared as a complex multiplet between δ 3.17 and 4.35. ^{13}C NMR (d_6-DMSO): δ 160.5 (C-2, 4, 6); 108.8 (C-1); *ca* 92 *br* (C-3, 5); 76.2, 71.7, 68.5, 67.8, 62.2 (allosyl carbons); 56.0 (2 and 6-OMe); 55.2 (4-OMe).

In some instances the *C*-alloside tetraacetate (**5**) was contaminated after prep. TLC with an equally mobile impurity (believed to be a disaccharide, cf. ref. [7]), which was removed by deacetylation and prep. TLC on silica gel, eluting with Me_2CO–EtOAc–H_2O (5:4:1) as described above.

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