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ALTERNANTHIN, A *C*-GLYCOSYLATED FLAVONOID FROM *ALTERNANTHERA PHILOXEROIDES*

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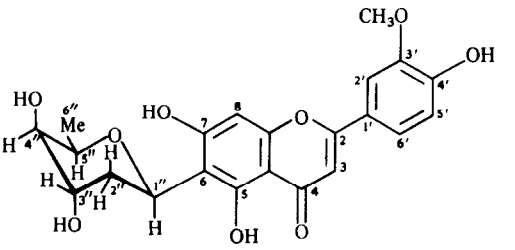
Abstract—The structure of alternanthin, a novel *C*-flavone glycoside from *Alternanthera philoxeroides*, has been deduced through the application of NOE difference and homonuclear correlation spectroscopy. The isolate contains the extremely rare sugar boivinose. A new method is described for the unambiguous assignment of the attachment of a sugar moiety to C-6 or C-8 of the flavone nucleus.

INTRODUCTION

Alternanthera philoxeroides Griseb. (Amaranthaceae) is native to the provinces around the Yangtze River and is used clinically in the People's Republic of China for the treatment of certain viral diseases, such as hepatitis, epidemic parotitis, hemorrhagic fever and influenza (unpublished results) Column chromatography of an extract of the stems and leaves on silica gel afforded α-sitosterol, β-sitosterol, stearic acid and a novel flavone alternanthin, whose structure elucidation is the subject of this communication.

RESULTS AND DISCUSSION

Alternanthin, $C_{22}H_{22}O_9$, mp 225–227°, $[\alpha]_D$ +86.2° (MeOH; *c* 0.23) gave a positive reaction with Mg/HCl and with ferric chloride, and its UV spectrum displaying λ_{max}270, 346 nm was typical for a flavonoid. No reaction was observed on treatment with refluxing dilute HCl and it was therefore concluded that alternanthin was a flavone *C*-glycoside. Treatment with acetic anhydride–pyridine at room temperature overnight afforded tetraacetate **2** and extending the reaction for a week yielded a pentaacetate **3**. Methylation [K_2CO_3, $(Me)_2SO_4$, acetone] afforded the trimethyl ether **4**. Alternanthin (**1**) therefore possesses three aromatic hydroxy groups, one of which is strongly hydrogen bonded, and two aliphatic hydroxy groups in the form of a dideoxy-hexose moiety Consequently, the structural issues to be resolved were the nature of the flavone, the nature and point of attachment of the sugar unit on the flavone nucleus and the stereochemistry and absolute configuration of the sugar moiety.



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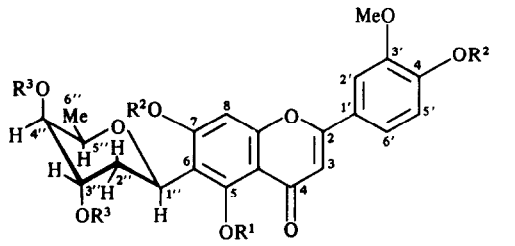

1 $R^1 = R^2 = R^3 = H$
2 $R^1 = H$, $R^2 = R^3 = Ac$
3 $R^1 = R^2 = R^3 = Ac$
4 $R^1 = R^2 = Me$, $R^3 = H$

Systematic studies of the UV spectrum (see Experimental) established the presence of phenolic hydroxy groups at positions 5, 7, and 4′, and the location of the methoxy group at the 3′-position in ring B was established through NOE difference spectroscopy where enhancement of an aromatic doublet at δ 7.58 ($J=2$ Hz) assigned to H-2′ was observed. A characteristic signal for H-3 of the flavone was observed at δ 6.60, indicating that the sugar unit must be attached at the C-6 or C-8 position

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of chrysoeriol [1]. Distinction between these two possibilities has, in the past, been made partly on the basis of UV spectral analysis [2, 3], and, in part, on the chemical shift changes induced in aromatic portons when phenolic hydroxy groups are acetylated [4]. Since it is known [2, 5] that the 5-methoxy group occurs at the most downfield position in a polymethoxylated flavone, the trimethyl ether derivative of alternanthin was prepared. Four singlet methyl groups were observed in benzene (δ 3.14, 3.33, 3.40 and 4.14), and irradiation of the latter signal resulted in an NOE enhancement in the aliphatic proton (1″-H) at δ 5.85. Consequently the sugar unit is located at the C-6 position of the flavone nucleus. Attention could then be focussed on the nature and stereochemistry of the hexose moiety.

The homonuclear COSY spectrum of alternanthin (**1**) obtained in DMSO-d_6 (Fig. 1) indicated the coupling of a three-proton doublet ($J = 6.0$ Hz) at δ 1.17 with a quartet at 4.04. In the delayed COSY spectrum this latter signal displayed weak coupling with the *ddd* system at δ 3.25 indicating that C-4″ is substituted by a hydroxy group, and that the coupling between 4″-H and 5″-H is less than 1.0 Hz. In the normal COSY spectrum, the signal at δ 3.25 also shows coupling with the attached hydroxy group proton (δ 5.06) and with an adjacent 3″-H (δ 3.86). This proton was also found to couple with its attached hydroxy group proton (δ 5.14) and with a methylene group (δ 1.50 and 2.21). From the magnitude of the coupling constants these protons could be assigned to the 2″-equatorial and 2″-axial protons, respectively. Each of these signals was also coupled with a broadened doublet of doublets at δ 5.33 ($J_1 = 12.3$ Hz, $J_2 = 3.1$ Hz) assigned to 1″-H, the anomeric proton, thereby establishing its axial orientation. The sugar unit is therefore unsubstituted at C-2 and C-6, and from the coupling constants, the 3″- and 4″-hydroxy groups were established to be *trans*-diaxial. Several of these ^{1}H ^{1}H coupling interactions were also confirmed through selective irradiation experiments. For example, irradiation of the *dd* at δ 1.50 (2″-H_a) resulted in simplification of the *ddd* at δ 2.21 (2″-H_e), and

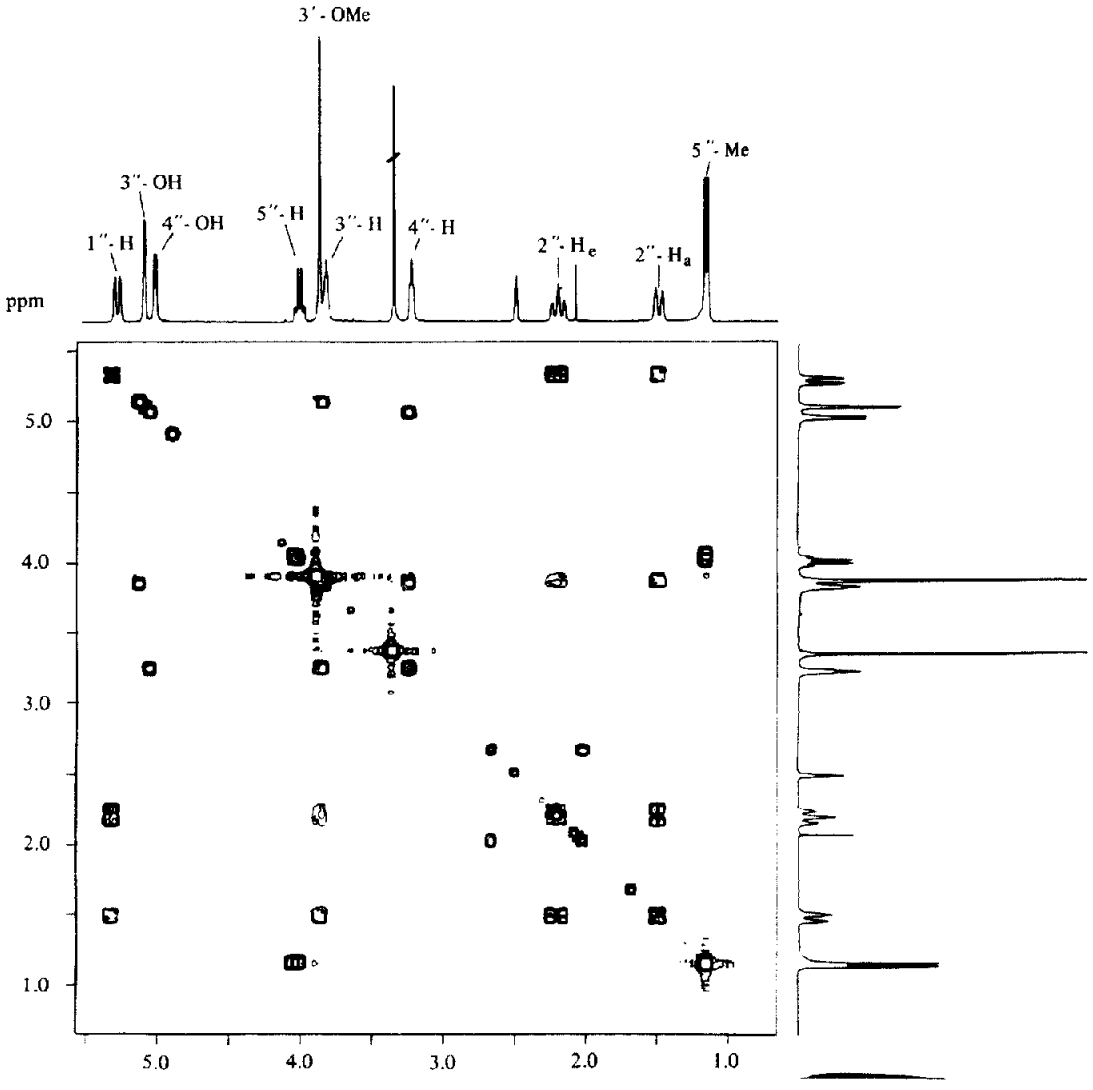

Fig. 1. ^{1}H–^{1}H COSY spectrum of alternanthin (**1**).

Table 1 Proton NMR assignments of alternanthin (1) and its derivatives 2-4

Proton	1*	2†	3†	4‡
3	6.60 (*s*)	6 54 (*s*)	6.58 (*s*)	6 61 (*s*)
8	6.95 (*s*)	6.92 (*s*)	7 22 (*s*)	6 79 (*s*)
2′	7 58 (*d*, 2 0)	7 39 (*d*, 2.1)	7 33 (*d*, 2 0)	7 33 (*d*, 2 0)
5′	6 94 (*d*, 7 5)	7 17 (*d*, 8.3)	7.11 (*d*, 8 0)	6 98 (*d*, 8 1)
6	7.58 (*d*), 7 5)	7.46 (*dd*, 8 3, 2 0)	7 09 (*dd*, 8 0, 2 0)	7 51 (*dd*, 8 1, 2 2)
1″	5.33 (*dd*, 12 3, 3 1)	5.27 (*m*)	5 03 (*m*)	5.51 (*dd*, 12 2 2.9)
2_a″	1 50 (*ddd*, 4 3)	n o	1.62 (*br d*, 14 0)	1 51 (*br d*, 14 7)
2_e″	2 21 (*ddd*, 14 0, 12 8, 2 7)	2.17 (*br d*, 14 0)	n.o.	2 77 (*ddd*, 14 5, 12.2, 2.4)
3″	3.86 (*ddd*, 3 4)	5.09 (*d*, 2.9)	5 03 (*d*, 3 0)	4.20 (*br s*)
4″	3 25 (*ddd*, 4 3)	4 85 (*d*, 3.4)	4 73 (*br s*)	3 40 (*br s*)
5″	4 04 (*q*, 6 0)	4 21 (*q*, 6 1)	3 99 (*q*, 6 0)	4.22 (*q*, 6.1)
6″	1 17 (*d*, 6 0)	1 27 (*d* 6.2)	1 10 (*d*, 6 0)	1.29 (*d*, 6 2)
OMe	3.90 (*s*)	3.93 (*s*)	3 87 (*s*)	3.14 (*s*), 3 33 (*s*), 3 40 (*s*), 4 14 (*s*)
OH	5 06 (*d*, 4.3), 5.14 (*d*, 3 5)	—	—	—
	9.79 (*s*), 9 97 (*s*)	—	—	—
OAc	—	2.17 (*s*), 2 20 (*s*)	2.09 (*s*), 2 13 (*s*), 2 29 (*s*)	—
	—	2.35 (*s*), 2.44 (*s*)	2.35 (*s*), 2 41 (*s*)	—

Multiplicity and coupling constants (Hz) are shown in parentheses
*Recorded in DMSO-d_6.
†Recorded in $CDCl_3$
‡Recorded in C_6D_6

Table 2 ^{13}C NMR assignments of alternanthin (1)*

C	Chemical shift
2	163 67
3	103 34
4	181 95
4a	103 00
5	157 17
6	110.26†
7	162.42
8	94 69
8a	156 03
1′	121.29
2′	110.03†
3′	150 69
4′	147 91
5′	115.67
6′	120.34
1″	70 53
2″	31 34
3″	67 33
4″	68.58
5″	66 41
6″	17.07
OMe	55 86

*Recorded in DMSO-d_6 at 90 8 MHz.
†Interchangeable

the *dd* at δ5.33 (1″-H_a) became a well-resolved doublet (J = 12.2 Hz) indicating the stereochemistry of H-1″ to be axial.

Although many of the coupling interactions were similar in the tetraacetate derivative **2**, H-5″ was found to show an additional coupling with a broadened doublet at δ4.85 (H-4″) which was attributed to a slightly altered conformation for the pyran ring induced by the two axial acetyl groups at C-3″ and C-4″ NOE studies on tetraacetate **2** also allowed the substitution on ring B to be confirmed. Thus irradiation at δ3.93 specifically enhanced the *meta*-coupled proton at δ7.39 supporting the substitution to be 3′–OMe, 4′–OH in ring B.

The data suggested a 2,6-dideoxy-hexopyranose sugar unit connected to the flavone moiety via a β (equatorial) linkage, and with the 3″- and 4″-hydroxy groups both in the axial orientation. There is no direct, positive evidence at the present time for the stereochemistry of C-5″ The methyl group is assigned to the β (equatorial) position on the basis of, (i) the absence of a NOE effect when either 5″–Me or 1″-H were irradiated, (ii) the absence of W coupling between 5″-H and 3″-H and (iii) the absence of a chemical shift change for 5″–Me on acetylation of 3″–OH.

According to the above data the sugar unit of alternanthin (**1**) is boivinose (2,6-dideoxy-*xylo*-hexose) which has previously been isolated from natural sources in both D- and L- enantiomeric forms [6]. The absolute configuration of the sugar was determined unambiguously from Hudson's isorotation rule [7, 8] and the NMR signals of the anomeric proton (1″-H) Since alternanthin (**1**) has a strong positive rotation, its hexapyranose sugar unit can be represented by either the α-D or β-L absolute configuration. Conformational analysis based on the previously described NMR evidence established the α (axial) position of 1″-H. Thus alternanthin (**1**) is chrysoeriol 6-*C*-β-L-boivinopyranoside. Comparison of the strong negative rotation of β-methyl-D-boivinopyranoside (−125° [9]) with that of alternanthin (**1**) also supported the assigned absolute stereochemistry for the sugar moiety of **1**. Alternanthin is the first flavonoid to be described containing a *C*-attached dideoxy sugar moiety [10].

EXPERIMENTAL

Mp uncorr NMR spectra were measured at 360 MHz using TMS as int standard Mass spectra were recorded at 70 ev

Plant material The stem and leaf material of *Alternanthera philoxeroides* was collected from the suburbs of Wuhan, Hubei Province, People's Republic of China A herbarium sample representative of the collection is deposited in the herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China

Isolation of alternanthin (1) Powdered stems and leaves of *Alternanthera philoxeroides* (20 kg) were extracted with 95% EtOH (3 × 60 l) under reflux The crude extract was evapd in *vacuo* to afford a residue (1 03 kg) A sample of the crude residue (100 g) was successively partitioned between 50% EtOH, petrol and $CHCl_3$ The $CHCl_3$ fraction was chromatographed on silica gel eluting with $CHCl_3$–EtOAc (1 1), to afford after recrystallization from MeOH, yellow needle-like crystals of alternanthin (1) (200 mg, 0 014%) having the following physical and spectroscopic properties mp 225 227 °, $[\alpha]_D$ +86 2° (MeOH, *c* 0 23), IR ν_{max}^{KBr} 3400–3100 *br*, 1615, 1575, 1520, 1430, 1070 cm^{-1}, UV λ_{max}^{MeOH} 271 (log ε 4 28), 346 (4 38), (MeOH + MeONa) 266, 410, (MeOH + $AlCl_3$) 280, 370, (MeOH + $AlCl_3$ + HCl) 280, 360, (MeOH + $MeCO_2Na$) 280, 360 nm, ^{1}H NMR, see Table 1, ^{13}C NMR, see Table 2, MS, *m/z* (rel int.) 430 (M^+, 9), 412 (7), 394 (2), 367 (7), 337 (100), 327 (28), 300 (16), 257 (1), 229 (1)

Alternanthin tetraacetate (2) Alternanthin (20 mg) was dissolved in Ac_2O (1 ml) and pyridine (2 drops) and the mixture allowed to stand at room temp overnight Work-up in the usual way afforded alternanthin tetraacetate (2) as white needle-like crystals, mp 152-155 °, IR ν_{max}^{KBr} 1735–1719 *br*, 1660, 1635, 1620 cm^{-1}, UV λ_{max}^{MeOH} 242 (4 30), 258 sh (4 23), 310 (4 27) nm, ^{1}H NMR, see Table 1, Mass measurement found 598 1858, calcd for $C_{30}H_{30}O_{13}$ 598 1886

Alternanthin pentaacetate (3) Alternanthin (30 mg) was dissolved in Ac_2O (1 ml) and pyridine (2 drops) and the mixture allowed to stand at room temp for one week Work-up in the usual way afforded alternanthin pentaacetate (3) as white needle-like crystals, mp 142 °, UV λ_{max}^{MeOH} 240 (4 27), 259 sh (4 22), 346 (4 29) nm, ^{1}H NMR, see Table 1, Mass measurement, Found 640 1802, calcd for $C_{32}H_{32}O_{14}$ 640 1813

Alternanthin trimethyl ether (4) Alternanthin (37 mg) was dissolved in a mixture of dry K_2CO_3 (1 5 g) in dried Me_2CO (5 ml) under N_2 Me_2SO_4 (0 5 ml) was added and the mixture refluxed for 18 hr The crude product was purified by prep TLC on silica gel to afford amorphous alternanthin trimethyl ether (4, 17 mg), UV λ_{max}^{MeOH} 242 (4 27), 329 (4 26) nm, ^{1}H NMR, see Table 1, ms, *m/z* 472 (M^+, 16), 457 (4), 455 (3), 413 (19), 395 (100), 369 (18), 355 (28), 341 (13), 313 (5)

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