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REEXAMINATION OF *GAILLARDIA AMBLYODON*: ISOLATION OF NEW PSEUDOGUAIANOLIDES*

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Abstract—Two new pseudoguaianolides, desacetylgaillardipinnatin and amblyodiol, have been isolated from *Gaillardia amblyodon* Gay in addition to the previously-found gaillardipinnatin and amblyodin. Structures have been established and the stereochemistry of amblyodin fully defined with the aid of NMR spectrometry at 270 mHz.

INTRODUCTION

As THE result of our earlier work on a small collection of *Gaillardia amblyodon* Gay,[1] we received additional plant material for chemical studies. This has led to the isolation of two new pseudoguaianolides, desacetylgaillardipinnatin (**1b**) and amblyodiol (**3a**), in addition to the previously-reported gaillardipinnatin[1,2] (**1a**) and amblyodin (**2b**).[1] We now report structures of the new compounds as well as our solution of the remaining problem in the amblyodin structure, the stereochemistry at C-9.

DISCUSSION

Desacetylgaillardipinnatin

The first new substance, m.p. 167–170°, $[\alpha]_D^{24}$ −523·5°, had IR bands (3440, 1770, 1748, 1700 and 1580 cm^{-1}) characteristic of an α,β unsaturated cyclopentenone, a conjugated lactone and a hydroxyl group. The empirical formula $C_{17}H_{20}O_6$ and MS measurement suggested the presence of an acetate. This was supported by the NMR spectrum (Table 1) which also indicated that the new substance might be a desacetyl derivative of gaillardipinnatin (**1a**) and a stereoisomer of amblyodin (**2b**, stereochemistry at C-9 undetermined). Confirmation for this belief was provided by acetylation of the new compound which gave a substance identical in all respects with **1a**.

The remaining problem was to decide between formulas **1b** and **1c**. The question was answered by an analysis of the NMR spectrum which, incidentally, provided convincing proof for the previously-suggested stereochemistry of **1a**. Irradiation at the frequency of H-7, identified in the usual fashion by decoupling H-13a and H-13b, collapsed a doublet at 4·15 ppm (simplified from a doublet of doublets by addition of D_2O and hence associated with a proton under carbon carrying a hydroxyl) to a singlet and a triplet centered at 4·55 ppm (H-8) to a doublet. The triplet at 4·94 ppm was unaffected. The results

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* Part XII in the series "Constituents of *Gaillardia* Species". For part XI see Ref. 1.

[1] HERZ, W. and SRINIVASAN, A. (1972) *Phytochemistry* **11**, 2093.

[2] HERZ, W., RAJAPPA, S., LAKSHMIKANTHAM, M. V. and SCHMID, J. J. (1966) *Tetrahedron* **22**, 693.

TABLE 1. NMR SPECTRA OF *Gaillardia amblyodon* CONSTITUENTS AND DERIVATIVES*

Compounds	H-1	H-2	H-3	H-6	H-7	H-8	H-9	H-10	H-13	H-14†	H-15†	Misc.
1a	3·13*ddd* (11, 3, 2)	7·72*dd* (6·5, 2)	6·16*dd* (6·5, 2)	5·64*d* (8)	3·2*m*	4·64*t* (9·5)	4·98*t* (9·5)	2·1*c*‡	6·20*d* (3·5) 5·89*d* (3·0)	1·24*d* (7)	1·27	2·16† (Ac) 1·95† (Ac)
1b	3·08*ddd* (11, 3·1, 1·9)	7·76*dd* (6·2, 1·9)	6·16*dd* (6·2, 3·1)	4·15*dd* (8·4, 2·4)	3·16*m*	4·55*t* (10)	4·94*t* (9·9)	2·09*c*‡	6·26*d* (3·4) 6·08*d* (3·2)	1·24*d* (6·5)	1·23	2·15† (Ac) 3·11 (*OH*)
2a	2·78*dt* (11, 2·7, 2)	7·58*dd* (6·2, 2)	6·17*dd* (6·5, 2·7)	5·94*d* (4·8)	3·4*m*	4·86*c*§	4·86§	2·13*c*‡	6·32*d* (3·5) 5·72*d* (3·2)	1·22*d* (6·5)	1·25	2·18† (Ac) 2·06† (Ac)
2b‖	2·77*dt* (11, 2·7, 2)	7·64*dd* (6·0, 2·0)	6·16*dd* (6, 2·7)	4·53*d* (4·5)	3·22*m*	4·81*t* (9·5)	4.88*t* (9·9)	2·21*c*‡	6·41*d* (3·7) 5·69*d* (3·1)	1·21*d* (6·4)	1·29	2·16† (Ac)
3a‖	2·83*dt* (11, 2·8, 2)	7·64*dd* (6, 1·6)	6·14*dd* (6, 2·4)	1·84*dd* (14·4, 8) 2·04*dd* (14·4, 8)	2·43*m*	4·8*c*§	4·8*c*§	2·11*c*§	3·68 (AB quartet, 12, 1·2) (2 protons)	1·16*d* (6·6)	1·20	2·18† (Ac)
3b	2·76*dt* (11, 2·8, 2)	7·64*dd* (6, 2)	6·16*dd* (6, 2·8)		2·53*m*	4·75*t* (10·0)	4·97*t* (9·9)	2·13*c*‡	3·68*br* (2 protons)	1·19*d* (6·5)	1·19	2·18†, 2·14 2·08† (Ac)
4	2·71*dt* (11, 2·8, 1·9)	7·61*dd* (6, 1·9)	6·17*dd* (6, 2·8)		2·31*m*	4·72*c*§	4·72*c*§	2·13*c*‡	4·39*d* (9) 3·87*d* (9)	1·18*d* (6·5)	1·18	2·15† (Ac) 1·52†, 1·47†
5•	2·67*dt* (11, 2·8, 1·5)	7·83*dd* (6, 1·5)	6·19*dd* (6, 2·8)			5·39*d* (9·5)	4·43*t* (10)	2·08*c*‡	—	1·06*d* (6·5)	1·10	2·12† (Ac)
6b						4·71*c*§	4·71*c*§		4·24*br* (2 protons)	1·02*d* (6)	1·03	2·17†, 2·13† 2·1† (Ac)

* Run at 90 MHz in $CDCl_3$ on a Bruker HFX NMR spectrometer, unless otherwise specified, using TMS as internal standard. Values are in ppm, multiplicities are indicated by the usual symbols: *d*-doublet; *t*-triplet; *m*-multiplet whose center is given, *c*-complex multiplet; *br*-broadened singlet. Unmarked signals are singlets. Figures in parentheses are coupling constants or line separations.

† Three protons.

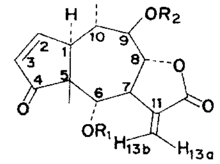
‡ Obscured partially by an acetate signal.

§ Center of AB system where A and B are each coupled to an additional proton.

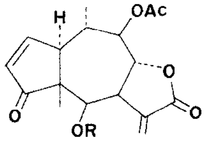
‖ Run at 270 mHz in $CDCl_3$ on a Bruker HX NMR spectrometer.

• In DMSO-d_6.

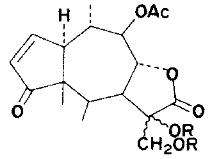
permitted assignment of the hydroxyl group to C-6 and the acetate to C-9. Other coupling constants enumerated in Table 1 were obtained from additional decoupling experiments and are consonant with the assigned stereochemistry.



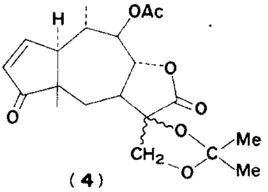
(**1**a) R_1, R_2 = Ac
(**1**b) R_1 = H; R_2 = Ac
(**1**c) R_1 = Ac; R_2 = H



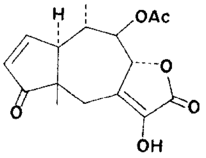
(**2**a) R = Ac
(**2**b) R = H



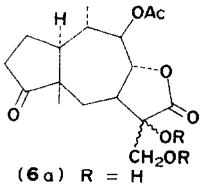
(**3**a) R = H
(**3**b) R = Ac



(**4**)



(**5**)



(**6**a) R = H
(**6**b) R = Ac

Amblyodin

As has been mentioned earlier,[1] the appearance of the H-8, H-9 multiplet of amblyodin at 60 or 90 mHz after irradiation at the frequencies of H-7 and H-10 did not permit a unique solution for the chemical shifts and coupling constants of H-8 and H-9, thus leaving the stereochemistry at C-9 undefined. The availability of additional quantities of amblyodin gave rise to the hope that oxidation of **2a** to a ketone or hydrolysis to **2b** might furnish substances more amenable to NMR analysis. However, experiments designed to lead to such compounds failed or gave complex mixtures. Use of NMR shift reagents likewise did not result in adequate separation of the H-8 and H-9 frequencies. Finally, determination of the NMR spectrum of **2a** at 270 mHz revealed a nicely-separated doublet of triplets due to the AB system of H-8 and H-9 (Table 1). The newly-determined large coupling constants ($J_{7,8} = 9{\cdot}5$, $J_{8,9} = 9{\cdot}5$ and $J_{9,10} = 9{\cdot}9$ Hz) can only be accommodated by a *trans*-diaxial relationship involving H-8, H-9 and H-10 which if added to previous knowledge of $J_{1,10}$ (11·2 Hz) and $J_{7,13}$ (> 3)[1] permits complete formulation of amblyodin as **2a**.

The CD curves of **1a**, **1b** and **2b** exhibit strong negative Cotton effects near 325 nm indicative of the *trans*-fused cyclopentenone system and absolute stereochemistry depicted in the formulas. The presence of this chromophore and the acetate functions is probably responsible for obscuring the Cotton effect due to the n,π^* transition of the α,β-unsaturated lactone function usually found near 250 nm.[3]

The chemical shift difference between H-13a and H-13b (geminal coupling <0·5 Hz) in **1b** and **2b**, which are C-6 epimers, is significantly different (0·18 vs 0·72 ppm, Table 1). Acetylation increases $\Delta\delta$ for **1a** somewhat (0·31), but not nearly to the point where it compares with $\Delta\delta$ in **2a** (0·60). Obviously, generalizations deduced for other classes of sesquiterpene lactones are not applicable to 6-hydroxypseudoguaianolides lactonized to C-8, as has already been pointed out earlier.[4]

Amblyodiol

The second new substance from *G. amblyodon*, m.p. 204–206°, $C_{17}H_{22}O_7$, was quite polar. UV and IR spectra coupled with the empirical formula indicated the presence of a cyclopentenone function (λ_{max} 222 nm, ε 8450, IR bands at 1710 and 1575 cm^{-1}), two hydroxyl groups (IR bands at 3490 and 3420 cm^{-1}), a saturated lactone and an acetate (IR band at 1772, shoulder at 1720 cm^{-1}). These deductions were confirmed by the NMR spectrum (Table 1) which exhibited the typical signals of the cyclopentenone moiety A, (H-2 and H-3, each coupled to H-1) and an acetate singlet, as well as a methyl doublet and a methyl singlet, but not the usual signals associated with H-13a and H-13b of an exocyclic methylene group. The presence of only one double bond was further established by hydrogenation to **6a**.

The NMR spectrum of amblyodiol also revealed an AB quartet at 3·68 ppm (after addition of D_2O, $J_{AB} = 12$ Hz) which was shifted downfield on conventional acetylation of amblyodiol and dihydroamblyodiol to diacetates containing altogether three acetate groups. Hence the grouping $R_3C\text{–}CH_2OH$, where $R \neq H$, was present. The absence of other paramagnetic shifts as the result of acetylation indicated that the second hydroxyl group was tertiary and, because of the facility with which it underwent acetylation, alpha to a carbonyl function, i.e. to the lactone group.[5] Hence a plausible partial structure was B.

Confirmation for the presence of α-glycol function B was provided by the formation of an acetonide **4** in whose NMR spectrum (Table 1) all signals except those of the

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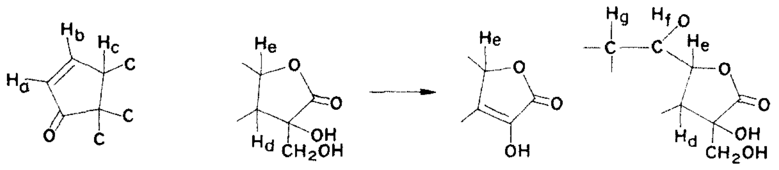
[3] Stöcklin, W., Waddell, T. G. and Geissman, T. A. (1970) *Tetrahedron* **26**, 2397.

[4] Yoshioka, H., Mabry, T. J., Irwin, M. A., Geissman, T. A. and Samek, Z. (1971) *Tetrahedron* **27**, 3317.

[5] Herz, W., Subramaniam, P. S. and Geissman, T. A. (1968) *J. Org. Chem.* **33**, 3743.

-CH_2OH group were essentially unchanged. Moreover, oxidation of amblyodiol with sodium metaperiodate resulted in conversion to a nor-enol lactone **5**, $C_{16}H_{18}O_6$ (λ_{max} 230 nm). The formation of **5** was accompanied by paramagnetic shift and simplification of a complex signal, previously found at 4·8 ppm, to a doublet at 5·39 ppm which in turn was coupled to a triplet at 4·43 ppm, an observation which permitted expansion of B to C



(A) (B) (C)

Although in the NMR spectrum of amblyodiol, the signals of H_e and H_f were superimposed, they were separated in the spectrum of **3b** (Table 1) into two triplets at 4·75 and 4·97 ppm (J 10 Hz) which were clearly spin-coupled to each other. Irradiation at 4·75 also affected a multiplet at 2·5 ppm (H_d) while irradiation at 4·97 also affected a multiplet at 2·1 ppm (H_g). Irradiation at 2·1 ppm (H_g) collapsed the methyl doublet of **3b** into a singlet and removed a large coupling from a multiplet at 2·76 ppm which was identified as H_c of A because irradiation at its frequency simplified the signals of H_a and H_b. Hence not only was the secondary methyl group attached to the carbon atom carrying H_g, but A and C could be combined as in **3a** (devoid of stereochemistry), with the quaternary methyl group attached to C-5 because of the multiplicity of H-1. This was confirmed by operating on amblyodiol at 270 mHz. Irradiation at the frequency corresponding to the multiplet of H-7, using the Fourier transform pulsing technique, led to identification of two doublets of doublets at 1·84 and 2·04 ppm as the signals of H-6a and H-6b and permitted determination of the remaining coupling constants in Table 1.

Construction of the Dreiding model of amblyodiol with a *cis*- and *trans*-fused lactone ring closed to C-8 revealed that the coupling constants were best satisfied by formula **3a** in which H-7, H-8, H-9, H-10 and H-1 are in a *trans*-diaxial relationship with each other. Since the CD curve of amblyodiol exhibits the strong negative Cotton effect characteristic of all other naturally occurring $\Delta^{2,3}$-4-keto-pseudoguaianolides, the ring fusion is *trans* with H-1 *alpha*. Parenthetically, this leads to a C-7 configuration identical with the C-7 stereochemistry of all other sesquiterpene lactones of established absolute configuration. The remaining problem, that of determining the stereochemistry at C-11, could not be solved satisfactorily. An attempt to use the method of Nakanishi *et al.*[6,7] for determining the configuration of acyclic diols failed when it was found that the CD curves of **3a** and **6a** after addition of $Pr(dpm)_3$ or $Eu(dpm)_3$ did not exhibit maxima near 310 and 290 nm.

EXPERIMENTAL

M.ps are uncorrected. Rotations were determined in $CHCl_3$, UV spectra in absolute MeOH, IR spectra as KBr pellets, CD curves in MeOH on a Jasco ORD/U recording spectrometer, MS on a high-resolution MS-902 mass spectrometer at 70 meV.

Extraction of Gaillardia amblyodon. (A) Dried and ground leaves of *G. amblyodon*, wt 880 g, collected by

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[6] NAKANISHI, K. and DILLON, J. (1971) *J. Am. Chem. Soc.* **93**, 4058.

[7] NAKANISHI, K., SCHOOLEY, D. A., KOREEDA, M. and DILLON, J. (1971) *Chem. Commun.* 1235.

Dr. W. P. Stoutamire in 1958 one mile South of Carrizo Spring, Dimmit Co., Texas (WPS No. 2694) was extracted and worked up in the usual manner.[8] The crude gum weighed 14 g. (B) Extraction of 540 g of *G. amblyodon*, collected by Dr. Stoutamire in 1960 on State Route 346, 9·8 miles north of its junction with State Route 476, Bexar County, Texas (WPS No. 3459) gave 12 g of crude gum. TLC of the crude extracts showed four major spots with identical R_f values (C_6H_6–EtOAc, 3:2). Hence the extracts were combined and chromatographed over 270 g of silicic acid (Mallinckrodt 100 mesh), 250 ml fractions being collected in the following manner: Fractions 1–24 (C_6H_6), 25–50 (C_6H_6–$CHCl_3$, 3:1), 51–75 (C_6H_6–$CHCl_3$, 1:1), 76–90 (C_6H_6–$CHCl_3$, 1:3), 90–100 ($CHCl_3$), 101–120 ($CHCl_3$–MeOH, 99:1), 121–132 ($CHCl_3$–MeOH, 3:1).

Fractions 26–30 eluted 100 mg of a solid which was recrystallized from EtOAc-hexane and shown to be identical with gaillardipinnatin (**1a**) by IR, NMR and TLC. Fractions 36–40 were combined and purified by preparative TLC. The major compound was recrystallized from EtOAc-hexane, yield 0·09 g, and had m.p. 167–170°, $[\alpha]_D^{24}$ −523·5° (*c* 0·042), IR bands at 3440 (–OH), 1770 and 1660 (α,β-unsatd. lactone), 1748 (acetate), 1700 and 1580 cm^{-1} (α,β-unsatd. cyclopentenone) (Found: C, 63·31; H, 6·49; O, 29·98; MW, 320·1255. Calc. for $C_{17}H_{20}O_6$:C, 63·74; H, 6·29; O, 29·97; MW, 320·1259, 18·2%). Other significant peaks in the high resolution MS were 302·1122 (12·6%, M–H_2O), 278·1155 (10·5%, M–C_2H_2O), 260·1054 (89·0%, M–$C_2H_2O_2$), 245·0804 (8·3%, M–$C_2H_4O_2$–Me), 242·0948 (30·9%, M–$C_2H_4O_2$–H_2O).

Fractions 55–70 eluted a mixture which was purified by repeated preparative TLC. The major component was identified as amblyodin (TLC, IR, NMR), yield 0·180 g, $[\alpha]_D^{24}$ −25.0° (*c* 0·12).

Fractions 102–116 gave a gummy solid which was purified by rechromatography over silica gel and preparative TLC. Recrystallization from EtOAc–C_6H_6 gave 0·8 g of amblyodiol, m.p. 204–206°, $[\alpha]_D^{24}$ −50·0° (*c* 0·08), IR bands at 3490 and 3420 (–OH), 1772 (lactone), 1720 sh (acetate), 1710 and 1575 cm^{-1} (α,β-unsatd. cyclopentenone), UV 222 nm (ε 8450) (Found: C, 60·35; H, 6·55; 0, 33·10; MW, 338 by chemical ionization.* Calc. for $C_{17}H_{22}O_7$: C, 60·35; H, 6·55; O, 33·10; MW, 338). The high resolution MS exhibited significant peaks at 308·1263 (39·5%, M–CH_2O), 278·1157 (10·7%, M–$C_2H_4O_2$), 250·1214 (23·6%, M–$C_2H_4O_2$–CO), 234·1260 (M–$C_2H_4O_2$–CO_2), 248·1060 (5·3%, M–$C_2H_4O_2$–CH_2O), 203·1067 (95%, M–$C_4H_7O_5$), 175·1122 (base peak, M–$C_5H_7O_6$).

All other fractions gave gums containing mixtures (TLC).

Acetylation of **1b**. Acetylation of 25 mg of **1b** with 0·5 ml Ac_2O and 1 ml pyridine in the usual manner furnished 16 mg of a diacetate, m.p. 255–260°, which was identical with gaillardipinnatin (**1a**) in all respects (TLC, IR, NMR).

Acetylation of amblyodin. Treatment of 50 mg **2b** with 0·5 ml Ac_2O and 1 ml pyridine gave a gum (**2a**) which was purified by preparative TLC and could not be induced to crystallize. (Found: C, 62·62; H, 6·26; O, 30·91. Calc. for $C_{19}H_{22}O_7$: C, 62·98; H, 6·12; O, 30·91%).

Acetylation of amblyodiol. Treatment of 30 mg **3a** with 0·5 ml Ac_2O and 1 ml pyridine gave 25 mg crude **3b** which was recrystallized from EtOAc-hexane and had mp 183–185°, IR bands at 1780 (lactone), 1755, 1740 (sh) (acetates), 1705 and 1575 cm^{-1} (α,β-unsatd. cyclopentenone) (Found: C, 59·56; H, 6·24; 0, 34·14; MW, 422·1583. Calc. for $C_{21}H_{26}O_9$; C, 59·71; H, 6·20; O, 34·09%%; MW, 422·1577). Other significant peaks in the high resolution MS were 467·1323 (3·2%, M–Me), 380·1454 (4·6%, M–C_2H_2O), 362·1356 (12·2%, M–$C_2H_4O_2$), 320·1263 (11·7%, M–C_2H_2O–$C_2H_4O_2$), 302·1157 (19·7%, M–$2C_2H_4O_2$), 276·1368 (40·1%, M–$C_5H_6O_5$), 260·1036 (62·5%, M–$2C_2H_4O_2$–C_2H_2O), 242·0941 (base peak, M–$3C_2H_4O_2$).

Acetonide (**4**). A mixture of 40 mg **3a**, 1·5 ml acetone, 90 mg anhyd. $CuSO_4$ and 2 drops of conc. H_2SO_4 was stirred at room temp. for 2 hr, mixed with solid $NaHCO_3$ and evaporated at reduced pressure. The residue was extracted thoroughly with $CHCl_3$. Evaporation of the washed and dried extract gave a gum which was purified by preparative TLC (C_6H_6–EtOAc, 7 : 3). The major component was recrystallized from EtOAc–hexane. yield 20 mg, m.p. 209–212 , IR bands at 1775. 1720, 1705 and 1575 cm^{-1} (Found: C, 63·31; H, 7·03; O, 29·91; MW, 378·1666. Calc. for $C_{20}H_{26}O_7$: C, 63·48; H, 6·93; O, 29·60%; MW, 378·1678).

Periodate oxidation of **3a**. To a soln of 50 mg **3a** in 3 ml MeOH was added a soln of 60 mg $NaIO_4$ in 4 ml (1 : 1) aq. MeOH with stirring. After 12 hr of stirring at room temp., the mixture was worked up by dilution with H_2O and extraction with EtOAc. The washed and dried organic layers were evaporated *in vacuo* and the residual **5** was recrystallized from Me_2CO, yield 30 mg, m.p. 252–255°, IR bands at 3250 (bonded –OH), 1760 (lactone) 1735 (acetate), 1690 and 1575 cm^{-1} (cyclopentenone), UV 230 and 275 nm (14700 and 3060) (Found: C, 62·25; H, 617; O, 31·20; MW, 306·1119. Calc. for $C_{16}H_{18}O_6$: C, 62·74; H, 5·92: O, 31·134%; MW, 306·1102). Other significant peaks in the high resolution MS were 264·0999 (base peak, M–C_2H_2O), 246·0903 (15·4%, M–$C_2H_4O_2$), 235·0966 (31·3%, M–C_2H_3O–CO), 218·0932 (32·8%, M–$C_2H_4O_2$–CO).

Hydrogenation of **3a**. A solution of 50 mg **3a** in 10 ml EtOAc was hydrogenated at atmospheric pressure with 35 mg of prereduced 10% Pb–C. Hydrogen uptake ceased after absorption of 1 mol equivalent of H. Filtration, removal of solvent and recrystallization from acetone furnished 40 mg **6a**, m.p. 222–224°, IR bands at 3480, 2410, 1775, 1740 and 1720 cm^{-1} (Found: C, 59·87; H, 7·05; O, 32·87; MW, 340·1507.

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* The parent ion was too weak to be determined by electron impact MS.

[8] HERZ, W. and HÖGENAUER, G. (1962) *J. Org. Chem.* **27**, 905.

Calc. for $C_{17}H_{24}O_7$: C, 59·99; H, 7·11; O, 32·90; MW, 340·1522). Other significant ions in the high resolution MS were 310·1418 (94·4%, M$-CH_2O$), 236·1415 (M$-C_2H_4O_2-CO_2$); 205·1229 (base peak, M$-C_4H_7O_5$), 178·1357 (41·0%, M$-C_5H_6O_6$); 177·1280 (60·9%, M$-C_5H_7O_6$). The diacetate **6b** was recrystallized from EtOAc–hexane, m.p. 206–208°. (Found: C, 59·02; H, 6·79; O, 34·16; MW, 424·1756. Calc. for $C_{21}H_{28}O_9$: C, 59·43; H, 6·65; O, 33·92%; MW, 424·1733). Other significant ions in the high resolution MS were 364·1512 (0·7%, M$-C_2H_4O_2$), 304·1306 (2·3%, M$-2C_2H_4O_2$) 278·1524 (5·3%, M$-C_5H_6O_5$).

CD curves. (0·4 mg/ml in MeOH): Gaillardipinnatin. $[\theta]_{375}$ 0; $[\theta]_{350}$ -2240; $[\theta]_{325}$ -4480 (min); $[\theta]_{300}$ -2240; $[\theta]_{275} = 0$; $[\theta]_{250}$ $+894$ (last reading). Desacetylgaillardipinnatin. $[\theta]_{375}$ 0; $[\theta]_{350}$ -1660; $[\theta]_{325}$ -3960 (min); $[\theta]_{300}$ -2110; $[\theta]_{265}$ 0 (max); $[\theta]_{244}$ -1780 (last reading). Amblyodin. $[\theta]_{380}$ 0; $[\theta]_{350}$ -1250; $[\theta]_{325}$ -2240 (min); $[\theta]_{300}$ -1250; $[\theta]_{277}$ -528 (max); $[\theta]_{257}$ -726 (min); $[\theta]_{250}$ 0; $[\theta]_{245}$ $+2510$ (last reading). Amblyodiol. $[\theta]_{375}$ 0; $[\theta]_{350}$ -1750; $[\theta]_{325}$ -2910 (min); $[\theta]_{300}$ -1450; $[\theta]_{262}$ 0 (max); $[\theta]_{250}$ -291; $[\theta]_{245}$ -678 (last reading).

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