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STRUCTURE OF CALLERYANIN AND ITS BENZYLIC ESTERS FROM *PYRUS* AND *PRUNUS**

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Abstract—The structure of calleryanin (3,4-dihydroxybenzyl alcohol 4-*O*-β-D-monoglucopyranoside), which occurs in *Prunus lusitanica* and many *Pyrus* species, has been confirmed by ^{1}H and ^{13}C NMR analysis and the structure of its benzylic esters with caffeic, protocatechuic and 4-hydroxybenzoic acids verified by catalytical hydrogenolysis. The occurrence of related compounds elsewhere in the plant kingdom is briefly reviewed.

INTRODUCTION

In a previous paper [1] the occurrence of the new glucoside calleryanin was reported in the leaf of *Pyrus calleryana* and *Prunus lusitanica*. Subsequent investigations [2–7] showed the caffeoyl ester of this glucoside to occur widely in the leaf and bark of the species of the genus *Pyrus*. In contrast, the 4-hydroxybenzoyl, protocatechuoyl and vanilloyl esters of calleryanin are found only in two closely related species of *Pyrus*, *P. calleryana* and *P. koehnei*, and in the leaf of the evergreen *Prunus lusitanica*. The occurrence of calleryanin, together with its benzylic esters, is of considerable phylogenetic and chemosystematic significance within the family Rosaceae: these distinctive phenolics occur here only in *Pyrus* (subfamily Maloideae) and in *Prunus* (subfamily Prunoideae), thus suggesting that a primitive member of the subfamily Prunoideae ($x = 8$) was involved in the allopolyploid ancestry of the subfamily Maloideae ($x = 17$), together with a primitive member of the subfamily Spiraeoideae ($x = 9$) [8].

Chemical studies (UV spectra, paper chromatography, colour reactions and mechanistic considerations) indicated that calleryanin (**1a**) is the 4-*O*-β-D-glucoside of 3,4-dihydroxybenzyl alcohol and that the esterifying acids are attached to the benzylic hydroxyl, but lack of material precluded a rigorous structural analysis, in particular of the position of attachment of the glucose molecule and the esterifying acids. Calleryanin and its esters have been re-isolated and further evidence for the structure of these compounds is now reported.

RESULTS AND DISCUSSION

The ^{1}H NMR spectrum of calleryanin (**1a**) showed three aromatic signals, at δ 7.06, 6.79 and 6.66, which were assigned on the basis of the observed ^{1}H–^{1}H splittings to the protons attached to C-5, C-2 and C-6, respectively. The aromatic protons of the model compounds **2a** and **3a** were less well resolved, but isovanillyl alcohol (**3a**) gave two signals in the ratio 1:2, whereas vanillyl alcohol (**2a**) gave an unresolved multiplet. This points to a greater similarity in the aromatic substitution pattern of calleryanin and **3a**, a conclusion which is supported by the ^{1}H NMR spectra of the TMSi ethers. The vanillyl derivative **2b** showed two groups of aromatic protons in the ratio 1:2, separated by 0.10 ppm, whereas both isovanillyl alcohol TMSi ether (**3b**) and the TMSi derivative **1b** showed only a broad signal at δ 6.72. The ^{1}H NMR data thus suggest that the site of attachment of the glucosyl moiety in **1a** is C-4 of 3,4-dihydroxybenzyl alcohol.

Confirmation of this structural assignment was obtained by ^{13}C NMR of **1a** and of some model compounds (Table 1). The observed chemical shifts for vanillin are in excellent agreement with those reported by Wenkert *et al.* [9], who completely assigned the spectrum using two- and three-bond carbon–hydrogen coupling information. Using their data and substituent chemical shifts [10], the chemical shifts for isovanillin were assigned. The spectra of vanillyl and isovanillyl alcohols were then calculated from those of the corresponding aldehydes and published data [11] for benzaldehyde and benzyl alcohol, and compared with the observed spectra to give the assignments shown in Table 1. All the aromatic carbon atoms of the glycoside **1a** have chemical shifts close to those of isovanillyl alcohol (**3a**), while the benzyl carbons of **1a** and **3a** have nearly the same chemical shift. The ^{13}C NMR spectrum of calleryanin thus strongly suggests a similar substitution pattern for the aglycone of **1a** and isovanillyl alcohol, and in conjunction with the ^{1}H NMR spectra confirms that the sugar residue is attached at C-4.

The earlier conclusion that **1a** is a β-glucoside [1] is confirmed by the observation of a large (6.5 Hz) ^{1}H–^{1}H splitting for the anomeric (C-1′) proton of the TMSi derivative **1b**; α-glycosides commonly have much smaller splittings (2–4 Hz) [12]. Further confirmation comes from ^{13}C NMR: the chemical shift of C-1′ occurs within the

* Part 8 in the series "Phenolic Compounds of the Genus *Pyrus*". For Part 7 see ref. [7].

Table 1. ^{13}C chemical shifts of vanillin, isovanillin, vanillyl alcohol, isovanillyl alcohol and calleryanin*

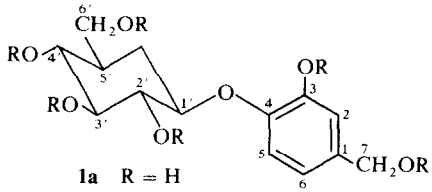
Assignment	Vanillin	Isovanillin	Vanillyl alcohol	Isovanillyl alcohol	Calleryanin
C-1	129.0	130.2	133.3	135.2	137.5
C-2	110.7	113.9	111.2	114.1	116.9
C-3	148.3	147.3	147.5	146.3†	146.8
C-4	153.3	153.5	145.2	146.2†	144.1
C-5	115.6	111.5	115.2	111.9	114.3
C-6	126.3	124.5	119.7	117.0	117.1
C-7	190.8	191.4	63.4	62.6	62.5
OMe	55.6	55.8	55.6	55.6	—
C-1′					102.8
C-2′					73.1
C-3′					77.1
C-4′					69.8
C-5′					75.8
C-6′					60.7

* 22.5 MHz, DMSO-d_6; ppm relative to TMS = 0.0.
† These assignments may be reversed.

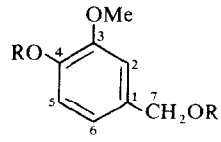
usual range for β-glucosides (102–105 ppm) [13], the α-anomers having chemical shifts below 101 ppm. The remaining glucosyl chemical shifts are very similar to the values reported for a number of β-glucopyranose derivatives [14], thus supporting a β-D-glucopyranoside structure for **1a**. Integration of the signals in the ^1H NMR spectrum of the TMSi derivative **1b** proves the sugar:aglycone ratio to be 1:1. On the basis of the ^1H NMR and ^{13}C NMR data, the structure of calleryanin is therefore 3,4-dihydroxybenzyl alcohol 4-*O*-β-D-mono-glucopyranoside.

Catalytic hydrogenolysis of the caffeoyl, 4-hydroxy-benzoyl and protocatechuoyl esters of calleryanin followed by paper chromatography of the products showed the presence of free dihydrocaffeic, 4-hydroxybenzoic and protocatechuic acids in the respective samples together with a second substance which appeared to be the same in each sample. Acid hydrolysis of this substance gave glucose together with an aglycone indistinguishable from 4-methylcatechol in colour reactions and PC in four different solvents; thus the common reduction product from the three esters must be the 1-glucoside of 4-methylcatechol as would be expected from their proposed structure. The formation of dihydrocaffeic acid rather than caffeic acid as the second product from caffeoylcalleryanin was investigated: the first stage in the hydrogenolysis appears to be reduction to dihydrocaffeoyl calleryanin, a small amount of which was isolated from a partially reduced sample and then split by further hydrogenolysis into dihydrocaffeic acid and the glucoside of 4-methylcatechol. It is thus concluded that the calleryanin esters involve the phenolic acid carboxyl and the benzylic hydroxyl of calleryanin.

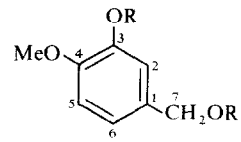
It has not been possible to perform a direct comparison between calleryanin and a compound of apparently identical structure which has been isolated from the



1a R = H
1b R = $SiMe_3$



2a R = H
2b R = $SiMe_3$



3a R = H
3b R = $SiMe_3$

cockroach, *Blaberus discoidalis* [15]. Their structural identification was by a very different approach. It is of interest to note that the biosynthesis of calleryanin in plants appears to differ radically from that in the cockroach and there is no apparent connection between plant and insect here. In the plant, calleryanin is probably biosynthesized in a manner analogous with that of salicin (the phenolic glucoside of 2-hydroxybenzyl alcohol), i.e. via a substituted cinnamoyl CoA ester [16, 17]. However, in the cockroach it is considered that 3,4-dihydroxybenzyl alcohol is formed via DOPA [15].

Caffeoyl- and protocatechuoylcalleryanin have also been found in the leaves of the gymnosperm *Podocarpus andina* (Podocarpaceae) [18] and 4-hydroxybenzoylcalleryanin, reported as an antifungal substance, has been found in the rootbark of the dicotyledon *Protea cynaroides* (Proteaceae) [19]. Lacticolorin, a glucoside which is equivalent to calleryanin with the glucoside moiety benzoylated at the 6-position, has been found in the leaves of *Protea lacticolor* [20]; it is accompanied in the plant by free 4-hydroxybenzoic, vanillic and protocatechuic acids and 3,4-dihydroxybenzyl alcohol, but calleryanin and its benzylic esters were not reported. A compound named pilorubrosin, similar to lacticolorin, but where D(+)-allose replaces the glucosidic moiety has been found in the leaves of *Protea rubropilosa* [21]. A substance named daphnoside, which has been assigned a tentative structure equivalent to the 3-methyl ether of calleryanin, has been found in the leaves of *Daphne mezereum* (Thymelaeaceae) [22]. An isomer of calleryanin, 2,3-dihydroxybenzyl alcohol 2-*O*-β-D-glucoside (idesin) has been reported from *Idesia polycarpa* (Flacourtiaceae) [23]; another isomer of calleryanin, the 2-*O*-β-D-glucoside of gentisyl alcohol (2,5-dihydroxybenzyl alcohol) has been found as the benzylic benzoyl ester (salireposide) in *Salix* [24]. Crenatin (3,4,5-trihydroxybenzyl alcohol 4-*O*-β-D-glucoside), which is equivalent to 5-hydroxycalleryanin, has been isolated from chestnut galls of *Castanea crenata* (Castaneaceae) [25]. Thus in the plant kingdom there exists benzyl alcohol itself, together with at least six different phenolic substitution patterns thereof, which are all glucosylated.

EXPERIMENTAL

Isolation of calleryanin and its esters. By PC on thick paper of EtOH extracts of leaves of *Prunus lusitanica* and *Pyrus calleryana* as previously described [1].

Reference compounds. The two isomeric vanillyl alcohols were prepared by the reduction of vanillin and isovanillin with $NaBH_4$ in aq. EtOH soln at room temp, recrystallized and their purity checked by PC in a range of solvents. TMSi ethers of the relevant compounds were prepared as described in refs. [26, 27].

^{1}H *NMR spectra.* (a) Hydroxy compounds (90 MHz, DMSO-d_6). Vanillyl alcohol (**2a**): δ 6.8–7.0 (3 H, *m*, aromatic), 4.46 (2 H, *s*, C-7), 3.87 (3 H, *s*, OMe); isovanillyl alcohol (**3a**): δ 6.95 (1 H, *m*, aromatic), 6.85 (2 H, *m*, aromatic), 4.57 (2 H, *s*, C-7), 3.87 (3 H, *s*, OMe); calleryanin (**1a**): δ 7.06 (1 H, *d*, J = 8 Hz, C-5), 6.79 (1 H, *d*, J = 2 Hz, C-2), 6.66 (1 H, *dd*, J = 8, 2 Hz, C-6), 4.62 (1 H, *m*, C-1′), 2.8–3.8 (6 H, *m*, glucosyl). (b) TMSi derivatives (100 MHz, CCl_4). Vanillyl alcohol TMSi ether (**2b**): δ 6.84 (2 H, *m*, aromatic), 6.74 (1 H, *m*, aromatic), 4.53 (2 H, *s*, C-7), 3.78 (3 H, *s*, OMe); isovanillyl alcohol TMSi ether (**3b**): δ 6.72 (3 H, *m*, aromatic), 4.53 (2 H, *s*, C-7), 3.78 (3 H, *s*, OMe); calleryanin TMSi ether(**1b**): δ 6.72 (3 H, *m*, aromatic), 4.90 (1 H, *d*, J = 6.5 Hz, C-1′), 4.52 (2 H, *s*, C-7), 2.9–3.9 (6 H, *m*, glucosyl).

^{13}C *NMR spectra* obtained (22.5 MHz, DMSO-d_6) under proton-decoupled conditions are tabulated in Table 1.

Hydrogenolysis. The calleryanin esters were split smoothly by H_2 in EtOH soln in the presence of Pd–C in a few hr at room temp. and pres. PC of the products was as previously described [1].

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