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A NOVEL STILBENE FROM BUD EXCRETION OF *ALNUS VIRIDIS*

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Key Word Index—*Alnus viridis*; Betulaceae; bud excretion; novel stilbene; 4′,5′-dihydroxy-3′-methoxy stilbene.

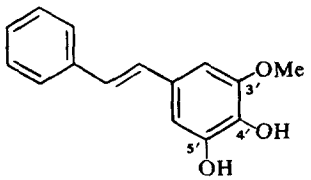
INTRODUCTION

Winter buds and catkins (male flowers) of *Alnus viridis* (Chaix) DC are covered with a whitish coating of lipophilic material which is excreted by capitate cells [1]. Such excretions are observed in many species of the genus and consist mainly of triterpenoids such as δ-amyrenone [2] but also contain several flavonoid aglycones. Among the latter, methyl ethers of apigenin, scutellarein, 6-hydroxykaempferol and quercetagetin predominate. By comparative TLC different species show individual flavonoid patterns [3]. Thus, *A. viridis* can be distinguished from all other *Alnus* species examined by the presence of galangin, galangin 3-methyl ether and 2′,4′-dihydroxy,6′-methoxy chalcone [4]. Furthermore, it shows a prominent UV-fluorescent light blue spot due to an unknown phenolic compound with two hydroxy groups [3]. We now wish to report its identification as 4′,5′-dihydroxy-3′-methoxy stilbene.

RESULTS AND DISCUSSION

The stilbene was present in several fractions of CC separation, but could not be crystallized. It was therefore acetylated to yield colourless crystals of the diacetate. This compound showed a weak M^+ at *m/e* 326 and successive loss of two acetyl units leading to a strong basic ion *m/e* 242, corresponding to $C_{15}H_{14}O_3$. The IR spectrum underlined the phenolic character of both acetyl groups (ν = 1770 cm^{-1}) and revealed the presence of a monosubstituted aromatic ring (ν = 692–755 cm^{-1}). PMR analysis confirmed the existence of two phenolic acetyl residues and showed one methoxyl group. Five protons at 7–7.45 ppm could be ascribed to the monosubstituted aromatic ring. One of the isochron hydrogen pairs must be attributed to a second aromatic ring carrying two acetoxy and one methoxy group; the other one would be situated on a vinyl residue. Hence the substance was assumed to be a stilbene.

Reduction of the acetate gave a dihydro-compound which had a weak M^+ at 328, and showed the presence of two acetyl units and three important ions at *m/e* 244, 153 and 91, the first showing reduction of a single double bond. The other two result from a homolytic cleavage between two $—CH_2—$, each attached to an aromatic ring (*m/e* 91 = phenyl-CH_2; *m/e* 153 = di-OH, mono-Me-phenyl-CH_2). These data fit well into the suggested stilbene structure. Furthermore, PMR confirmed this proposal: the reduced derivative showed a new signal at 2.88 ppm, corresponding to four protons ($—CH_2—CH_2—$) and two doublets at 6.44 and 6.53 ppm, relative to two protons of the aromatic B ring. This latter information was particularly valuable in assigning the aromatic substitution pattern. Indeed, PMR of the original acetylated compound with two singlets, one for the acetyl groups, the other for the aromatic hydrogen of the B nucleus, indicated the existence of a symmetric substitution: H-2′, H-6′, 3′,5′-diacetoxy, 4′-monomethoxy. However, PMR of the reduced compound showed that both aromatic protons appeared as two doublets with a coupling constant of J = 2.5 Hz, characteristic of two protons in meta-position with an assymmetric environment, i.e. 4′,5′-diacetoxy,3′-monomethoxy substitution (4′,6′-diacetoxy,2′-monomethoxy structure would have led to the presence of separated signals for each $—CH_2—$). If the protons of the B nucleus appeared isochronous in the unreduced substance, this is due to anisotropy of the double bond, the effect of which became predominant over the dissymmetry of the aromatic substitution. Structural investigation by the usual physicochemical techniques thus established the compound isolated from buds of *Alnus viridis* to be 4′,5′-dihydroxy, 3′-monomethoxy stilbene (**1**). The *trans* configuration was supported by the strong IR band at 970 cm^{-1}.



From excretions of *Alnus sieboldiana* Asakawa [5] had found, along with cinnamic acid derivatives, *trans*-stilbene itself, and from *A. pendula* excretions he isolated pinosylvin and pinosylvin monomethyl ether [6] (together with the corresponding flavanones, pinocembrin and pinostrobin). The stilbene described here from *A. viridis*, however, is a novel compound in its class. Its structure is remarkable because stilbenes are built up in general by condensation of one molecule of a cinnamic acid with three acetic acid units. Hence, most naturally occurring stilbenes show a 2′,4′-meta-diOH substitution in one ring and a substitution pattern of (hydroxylated) cinnamic acids in the other. One known exception of this rule is 4-hydroxystilbene. The formation of the compound (**1**) must be due to secondary hydroxylation like that leading to the 6-hydroxy flavonoids found previously [3].

EXPERIMENTAL

The lipophilic material covering the winter buds of *A. viridis* (collected in the Jura mountains near Grenoble, France) was extracted with Me_2CO at room temp. The soln was evapd and the residue taken up in C_6H_6. Separation was effected on columns of Si gel, eluted with C_6H_6 with increasing quantities of EtOAc and MeOH. The fractions containing the blue fluorescent compound produced a solid after several days but

this could not be crystallised. It was acetylated (Ac_2O–Py) and the product gave colourless crystals, mp 133°. UV: λ_{max}^{MeOH}: 326 (*sh*), 312, 302, 236 (*sh*), 228 (*sh*) nm. IR ν_{max}^{KBr}: 692, 745, 755, 817, 850, 870, 900, 1010, 1080, 1140, 1200, 1270, 1300, 1340, 1370, 1420, 1450, 1470, 1500, 1600, 1770, 300 cm^{-1}. PMR (250 MHz, CCl_4, TMS) δ: 2.21 (6 H, *s*), 3.85 (3 H, *s*), 6.85 (2 H, *s*), 6.94 (2 H, *s*), 7–7.45 (5 H, *m*) ppm. MS 70 eV *m/e* (rel. int.): 326 (11), 284 (36), 242 (100; 242.0944, calc. 242.0943), 227 (0.5), 223 (1), 209 (4), 181 (8), 165 (3), 153 (4), 152 (5), 141 (4), 115 (3), 91 (3), 84 (7), 69 (5), 58 (7), 65 (7), *m** 247.4 (326–284), 206.2 (284–242), 156.8 (209–181), 129.3 (181–153), 93.8 (141–115). Reduction by hydrogenation with Pd–C catalyst in EtOH and purification by Si gel column chromatography with $CHCl_3$ as eluent. PMR δ: 2.20 (6 H, *s*), 2.88 (4 H, *s*), 3.73 (3 H, *s*), 6.44 (1 H, *d*, J = 2.5 Hz), 6.53 (1 H, *d*, J = 2.5 Hz), 7.04–7.30 (5 H, *m*) ppm. MS *m/e*: 328 (8), 286 (30), 244 (56), 195 (4), 153 (100), 138 (2), 91 (38), 71 (8), 57 (16). *m* 249.4 (328–286), 208.2 (286–244), 95.9 (244–153), 124.5 (153–138).

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A NEW CHALCONE GLUCOSIDE AND ISOSALIPURPOSIDE FROM *ACACIA CYANOPHYLLA*

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Key Word Index—*Acacia cyanophylla*; Leguminosae; 4,2′,4′,6′-tetrahydroxychalcone 4-glucoside; isosalipurposide.

Quercetin 3-*O*-glucoside has previously been reported from the leaves of *Acacia cyanophylla* [1]. The present paper describes the isolation of two anthochlor pigments, isosalipurposide and chalcononaringenin 4-glucoside, from the flowers of this plant. These pigments are primarily responsible for the yellow flower colour.

Prep PC of an ethanolic extract of the flowers of *Acacia cyanophylla* afforded two anthochlor pigments (C_1 and C_2). C_1 was identified as isosalipurposide (4,2′,4′,6′-tetrahydroxychalcone 2′-glucoside) by UV spectral analysis in the presence of usual shift reagents [2, 3] and paper co-chromatography with authentic material (5 solvents). The above identification was confirmed by controlled acid hydrolysis, total acid hydrolysis and treatment with β-glucosidase to give naringenin and D-glucose, and by conversion (by heating in a NaOAc soln [4]) to naringenin 5-*O*-glucoside. This is the first report of isosalipurposide in the Leguminosae.

The UV spectrum of C_2 showed λ_{max}^{MeOH} 368 nm and a bathochromic shift (50 nm) with both $AlCl_3$ and $AlCl_3$/HCl which is consistent with a chalcone skeleton with a hydroxyl group at position 2′. Since the UV spectrum showed a bathochromic shift (50 nm) in the presence of NaOMe without any increase in peak intensity [3], this chalcone lacks a free 4-hydroxyl group. The presence of a 4′-hydroxyl group was indicated by a bathochromic shift (10 nm) with NaOAc [3]. When heated in NaOAc [4] soln C_2 easily isomerized to the corresponding flavanone (F). The UV spectrum of F showed λ_{max}^{MeOH} 289 and 324 nm (*sh*) and bathochromic shifts with NaOAc (37 nm), $AlCl_3$ (21 nm) and $AlCl_3$/HCl (21 nm). The above spectral data are consistent [3] with those of a 5,7-dihydroxy flavanone. Treatment with β-glucosidase, controlled acid hydrolysis and total acid hydrolysis of F gave naringenin and D-glucose. The aglycone: sugar ratio was 1:1.09. Methylation of F followed by acid hydrolysis gave naringenin 5,7-dimethyl ether (characterized by alkaline degradation which gave *p*-coumaric acid and di-*O*-methylphloroglucinol) and 2,3,4,6-tetra-*O*-methyl-D-glucose. The PMR spectrum [3] of F (TMS ether, 60 MHz, CCl_4) showed a typical four peaks pattern of two doublets (each J = 8.5 Hz) at δ 7.12 (C-2′ and C-6′ protons) and 6.78 (C-3′ and C-5′ protons) for a 4′-oxygenated B ring, an ABX pattern characteristic of the protons at C-3 (AB) and C-2 (X) of a flavanone nucleus [3], two doublets (δ-5.95 and 6.05; each J = 2.5 Hz) for C-6 and C-8 protons, a doublet (δ 5.0; J = 7 Hz) for C-1″ proton and a signal between 3.8 and 3.0 δ (glucosyl 6 protons). Thus F is the flavanone naringenin 4′-*O*-glucoside and C_2 must be 4,2′,4′,6′-tetrahydroxychalcone 4-glucoside which has not been previously described. The occurrence of 2′,6′-dihydroxychalcones in nature is somewhat rare, possibly because of their ready conversion to the corresponding flavanones which are stabilized due to the hydrogen bonding between the 5-hydroxyl and the 4-carbonyl groups.

EXPERIMENTAL

Isolation of the pigments. Fr. flowers of *Acacia cyanophylla*, collected in Catania, were homogenized and extracted 3 × with