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PHYLLOFLAVAN, A CHARACTERISTIC CONSTITUENT OF *PHYLLOCLADUS* SPECIES

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Key Word Index—*Phyllocladus alpinus*; Podocarpaceae; catechin; epicatechin, phylloflavan, epimerization, configuration.

Abstract—Selective visualization of 2D TLC of extracts of *Phyllocladus* species with vanillin–hydrochloric acid showed a characteristic pattern of constituents attributable to catechin, epicatechin and phylloflavan, a new flavanoid compound. Optical rotation measurements showed that while catechin and epicatechin are of the normal type with the 2*R* configurations, the flavan moiety in phylloflavan is of the opposite 2*S* configuration. Spectroscopic data of the new compound and its hydrolysis products showed phylloflavan to be *ent*-epicatechin-3-*δ*-(3,4-dihydroxyphenyl)-*β*-hydroxypentanoate.

INTRODUCTION

The genus *Phyllocladus* comprises six species of evergreen trees which are limited in their geographical distribution. Half of these species are confined to New Zealand. An interesting feature of this genus is the lack of morphological specialization of the leaves which are actually flattened branchlets or cladodes which function as leaves. The flavan TLC pattern is very distinctive, the ethyl acetate extracts, when examined by 2D TLC and visualized selectively with vanillin–hydrochloric acid reagent, revealed only three major components; catechin, epicatechin and an unknown constituent named here as phylloflavan. The relative proportion of these constituents, as estimated by colour intensity on visualization with vanillin–hydrochloric acid on 2D TLC, varied among species and also within species obtained from different habitats. In this sample of *P. alpinus* the proportion of the new flavanoid to catechin and epicatechin was *ca* 4:1:1, respectively. Quantitative separation of phylloflavan from the simple flavans was readily effected by chromatography on Sephadex LH-20.

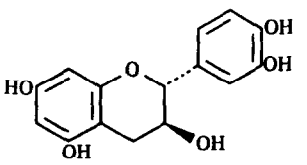
RESULTS AND DISCUSSION

Phylloflavan possesses a carbonyl function as evident from its IR absorption at 1710 cm^{-1} and ^{13}C NMR chemical shift at *δ* 171.3. Alkaline hydrolysis with aqueous methanolic potassium hydroxide gave catechin, epicatechin and a carboxylic acid. ^{13}C NMR studies employing gated spin-echo (GASPE) and coupling techniques showed the acid product to possess a carbonyl carbon chemical shift at *δ* 176.6, three quarternary aromatic carbons at *δ* 145.3, 143.3 and 135.1 with the remaining unsubstituted aromatic carbons at *δ* 121.0, 116.9 and 116.8, a tertiary oxygenated carbon at *δ* 68.6 and three methylene carbons at *δ* 43.0, 29.3 and 31.5 (Table 1). From these data it may be concluded that the aromatic ring is that of a catechol function with an aliphatic carboxylic chain located in the *para* position to one of the hydroxyls. The ^1H NMR spectrum of this product (prior exchanged once with deuterated water) aided by double irradiation experiments showed one methine proton at *δ* 3.76 as a multiplet brought about by couplings with the two groups of methylene protons at *δ* 1.60 and 2.27. While the chemical shift at *δ* 2.27 showed proton couplings with the methine proton only, the signal at *δ* 1.60, in addition, showed further coupling with another set of methylene protons at *δ* 2.45. The aromatic proton signals at *δ* 6.25–6.70 confirmed the presence of the catechol functionality with characteristic *ortho* couplings between the H-5^1 and H-6^1 protons and *meta* couplings between H-6^1 and H-2^1 protons. The mass spectrum of this product showed the $[M]^+$ at *m/z* 226 which, together with the NMR data, is fully consistent with the structure of *δ*-(3,4-dihydroxyphenyl)-*β*-hydroxypentanoic acid (**3**). The assignment of the hydroxyl group to the *β*-carbon was made by the operation of Schoolery's rule [1], the benzylic methylene protons being more downfield at *δ* 2.45 than the methylene protons *α* to the carboxylic acid at *δ* 2.27 As the methine proton was coupled with the methylene protons of the *α*-carbon at *δ* 2.27, the hydroxyl group must, therefore, be located at the *β*-carbon as assigned in **3**. This chemical structure may also be rationalized on biosynthetic grounds; the compound may be visualized as being formed from caffeic acid by condensation with acetyl-CoA and the resulting *α*,*β*-unsaturated ketone subsequently reduced to the saturated alcohol.

The production of both catechin and epicatechin on hydrolysis of phylloflavan is not unexpected as catechin (2*R*, 3*S*) and epicatechin (2*R*, 3*R*) readily and reversibly epimerize to *ent*-epicatechin (2*S*, 3*S*) and *ent*-catechin (2*S*, 3*R*), respectively, under alkaline conditions [2]. The 5:1 ratio of catechin–epicatechin, as observed in the hydrolysis sample, is also consistent with the equilibrium levels of the 2,3-*trans*–2,3-*cis* compounds during epimerization. In addition, both the catechin and epicatechin are dextrorotatory, i.e. the former has the normal 2*R* configuration while the latter has the abnormal 2*S* configuration which is evidence of the chemical interconversion

Table 1. ^{13}C NMR chemical shifts (δ-values) obtained in acetone-d_6–water (1:1)

Compound	C-2	C-3	C-4	C-4a	C-6	C-8	C-1′	C-2′	C-5′	C-6′	C-3′ C-4′	C-5 C-7 C-8a	α-C	β-C	γ-C	δ-C	C-1″	C-2″	C-5″	C-6″	C-3″ C-4″	C=O
Catechin	82.0	67.9	28.1	100.9	96.7	95.7	131.5	115.6	116.6	120.3	145.4 145.5	156.3 156.7 156.9	—	—	—	—	—	—	—	—	—	—
Epicatechin	79.1	66.8	28.6	100.3	96.8	96.0	131.8	115.4	116.4	119.6	145.0 145.2	156.6 156.6 157.1	—	—	—	—	—	—	—	—	—	—
δ-(3,4-Dihydroxyphenyl)-β-hydroxypentanoic acid (ex. phylloflavan)	—	—	—	—	—	—	—	—	—	—	— —	— —	39.3	68.6	31.5	43.0	135.1	116.8	116.9	120.3	143.3 145.3	176.6
Phylloflavan	78.2	69.8	28.5	98.6	96.5	95.2	130.7	115.2	115.9	118.7	145.3 145.3	155.7 156.7 157.5	39.2	67.7	31.2	42.7	134.7	114.4	115.6	120.1	143.4 114.8	171.3



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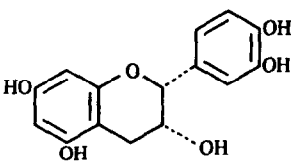

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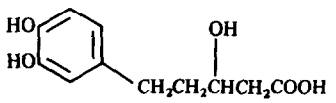

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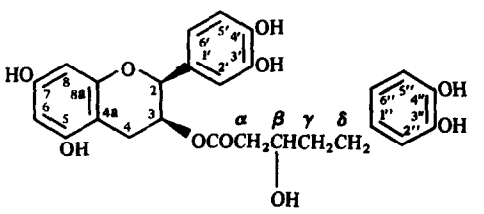

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which has taken place. The question concerning which of these flavans is represented in phylloflavan is resolved by examination of the ^{13}C NMR spectrum. The chemical shift at δ78.2 attributed to the C-2 of the flavan moiety is indicative of the C-2 aryl and C-3 hydroxyl substituents being *cis* to each other. Furthermore, the slight upfield shift of the C-2 and the downfield shift of the C-3 chemical shifts observed for phylloflavan with respect to the corresponding epicatechin carbon chemical shifts are analogous to those observed for an epicatechin moiety with a gallate substituent on C-3 [3]. This characteristic constituent of the *Phyllocladus* sp. is, therefore, *ent*-epicatechin-3-δ-(3,4-dihydroxyphenyl)-β-hydroxypentanoate (**4**). The proton chemical shifts of phylloflavan are now readily rationalized by the incorporation of the relevant proton chemical shifts values of epicatechin 3-gallate [4] and of δ-(3,4-dihydroxyphenyl)-β-hydroxypentanoic acid (**3**).

Naturally occurring flavans normally possess the 2*R* configuration but the abnormal 2*S* configuration has also been found a number of times [5, 6]. This phenomenon of enantiomerism, in fact, forms the distinctive feature of the catechins and the proanthocyanidins of the Monocotydae [7, 8]. However, the presence of a contrasting configuration at C-2 between the free flavans (2*R*) and the flavan moiety (2*S*) in phylloflavan is unusual. An analogous example of this contrasting stereochemistry has been reported in the bark of *Rhaphiolepis umbellata* where catechin-5-*O*-glucoside possessed the normal 2*R* configuration and catechin-7-*O*-glucoside had the abnormal 2*S* configuration [9]. It is apparent from this heterogeneity of configuration in the epicatechin structure that phylloflavan is unlikely to be formed by direct esterification of the catechins which co-occur with it. Also, the absence of the related catechin analogue of phylloflavan in the plant extracts suggests that these free flavans are not closely related biosynthetically to the ester and that the *ent*-epicatechin moiety in phylloflavan is likely to be synthesized quite independently from them.

EXPERIMENTAL

IR spectra were measured in KBr pellets. Specific rotations were obtained at ambient temp TLC was carried out on Schleicher and Schull cellulose plates using *t*-BuOH–HOAc–H_2O (3:1:1, solvent A) and HOAc–H_2O (6:94, solvent B) and visualized by spraying with vanillin–HCl reagent. A voucher specimen of *P. alpinus* is deposited in the Herbarium, Botany Division, Christchurch, New Zealand (CHR 388234).

Extraction. Ground up twigs and leaves (100 g) of *P. alpinus* were left to soak in MeOH–H_2O (1:1) overnight (3 × 350 ml). The combined extracts were concd to half vol. under red. pres. and to this residue was added an equal vol. of H_2O. The resulting aq. extract was washed with $CHCl_3$ (3 × 150 ml) and then exhaustively extracted with EtOAc (4 × 150 ml). The EtOAc extracts were combined and concd, the residue dissolved in EtOH–H_2O (1:1) and the soln placed over a column of Sephadex LH-20. Fractions (20 ml) were collected and monitored by TLC Tubes 32–45 were found to contain a mixture of catechin [R_f 0.65 (A); 0.50 (B)] and epicatechin [R_f 0.55 (A); 0.35 (B)]. Confirmation of their identity was by ^{13}C NMR of the mixture and the relative chemical shift intensity of their respective carbons indicated they occurred in a *ca* equal concn as was also shown by the comparable colour intensity of the TLC spots. The epimers were resolved by chromatography on Sephadex LH-20 using EtOH–H_2O (3:17) which yielded (−)-epicatechin and (+)-catechin in that order.

Phylloflavan [ent-*epicatechin*-3-δ-(3,4-*dihydroxyphenyl*)-β-*hydroxypentanoate*] (**4**). Collection of fractions from the chromatography of the plant extract, as described above, was continued and tubes 60–100 were shown by TLC to contain a third new compound. Concn of this fraction gave 0.8 g of residue which was reprocessed on the Sephadex column using EtOH–H_2O (1:1) to give chromatographically homogeneous phylloflavan, $[\alpha]_D^{578}$ −7° (MeOH; 0.02); R_f (A) 0.75; R_f (B), 0.20. IR ν_{max}^{KBr} cm^{-1}: 820, 1105, 1140, 1285, 1445, 1515, 1610, 1710, 3200–3500. ^1H NMR (Me_2CO-d_6): δ1.65 (*m*, γ-H), 2.38 (*d*, α-H), 2.45–3.10 (*m*, δ-H, H-4), 3.90 (*m*, β-H), 4 98 (*d*, H-2), 5.22 (*m*, H-3), 5.95 (*d*, H-6), 6.07 (*d*, H-8), 6.50–6.90 (*m*, catechol ring H) and 7.6–8.3 (–OH protons).

Hydrolysis. Phylloflavan (200 mg) was dissolved in 2.5% KOH in EtOH–H_2O (1:1) under N_2 and the reaction mixture left at room temp. overnight. The resulting dark coloured soln was dil. with 1 vol. H_2O and acidified with 10% H_2SO_4 and the products extracted with EtOAc (4 × 50 ml). The combined EtOAc extracts were concd and the residue subjected to chromatography using Sephadex LH-20 with EtOH–H_2O (1:1).

δ-(3,4-*Dihydroxyphenyl*)-β-*hydroxypentanoic acid* (**3**). This

compound was the first to be eluted $[\alpha]_D + 13°$ (MeOH; *c* 0.01); R_f (A), 0.85; R_f (B) 0.80. ^{1}H NMR (DMSO-d_6): δ1.58 (*m*, γ-H), 2.28 (*d*, α-H), 2.45 (*t*, δ-H), 3.76 (*m*, β-H) and 6.25–6.75 (*m*, aromatic H) ^{13}C NMR (Me_2CO-d_6–H_2O, 1:1): δ31.5, 39.3, 43.0, 68.6, 116.8, 116.9, 121.0, 135.1, 143.3, 145.3 and 176.6.

A later fraction contained a mixture of catechin and epicatechin which was separated by rechromatography on Sephadex using EtOH–H_2O (3:17). Eluted first was epicatechin, $[\alpha]_D^{578} + 38°$, followed by catechin, $[\alpha]_D^{578} + 1.5°$

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