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SULPHATE ESTER OF *TRANS*-4-HYDROXYPIPECOLIC ACID IN SEEDS OF *PELTOPHORUM*

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Key Word Index—*Peltophorum africanum*; Leguminosae; *trans*-4-hydroxypipecolic acid-4-sulphate; FAB-MS; EIMS; ^{13}C NMR; ^{1}H NMR; non-protein amino acid; sulphate ester; chemotaxonomy.

Abstract—An acidic compound isolated from seed of the legume *Peltophorum africanum* has been characterised on the basis of FAB-MS, EIMS, ^{13}C and ^{1}H NMR as *trans*-4-hydroxypipecolic acid-4-sulphate. This is the first naturally occurring sulphate ester of a non-protein amino acid to be described. The possible systematic significance of the distribution of the ester within *Peltophorum* and related genera is considered.

INTRODUCTION

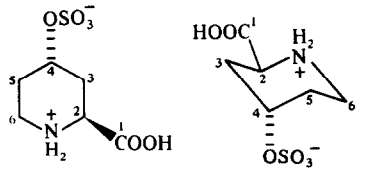
The legume genus *Peltophorum* [1] comprises seven to nine species of trees extending thinly and disjunctly throughout the tropics from Brazil and the W. Indies through S. Africa to S. Asia and Australia. Taxonomically the genus is at present considered to be at the centre of a group of small discrete Caesalpinoid genera, which when amalgamated form a series in the elaboration of flower structure [1].

Many species of Leguminosae contain non-protein amino acids in their seeds and the accumulation of one or more of these compounds may be characteristic of a particular taxon, usually a genus or subgenus [2]. A previous extensive survey of non-protein amino acids in seeds of Caesalpinieae [3] has shown that those of certain *Peltophorum* species accumulate a strongly acidic compound reacting with ninhydrin reagent to give a characteristic green colour. We now report the isolation of this compound from *Peltophorum africanum* and its characterisation as *trans*-4-hydroxypipecolic acid-4-sulphate. The distribution of this compound in various collections of *Peltophorum* and some related genera has also been studied.

RESULTS AND DISCUSSION

The unknown compound designated PA1 was isolated from aqueous MeOH extracts of *Peltophorum africanum* seed by ion exchange chromatography and was crystallised as the free base. Elemental analysis was consistent with the molecular formula $C_6H_{11}N_1S_1O_6$ (see Experimental). The IR spectrum indicated the presence of a COOH group (1720 cm^{-1}) and the absorptions at 1250 cm^{-1} and 855 cm^{-1} were consistent with the presence of an axial sulphate of a secondary alcohol [4]. The positive FAB-mass spectrum showed after preprotonation with oxalic acid, $[MH]^+$ (*m/z* 226, 100%), an isotope pattern clearly indicating the presence of one S atom. The major fragmentation resulted from the losses of SO_3, (*m/z* 146, 18%), and H_2SO_4 (*m/z* 128, 14%). This M_r was confirmed by the negative FAB-mass spectrum of $[MH]^-$ (*m/z* 224, 100%) with a monosulphur isotope pattern and the only fragment ion (*m/z* 97, 80% $[HSO_4]^-$) provided good evidence for the presence of a sulphate ester. In contrast, the high temperature (220°) EI-mass spectrum showed the presence of both a carboxylic acid (*m/z* 44, 25%, $[CO_2]^+$) and a sulphate ester (*m/z* 64, 100%, $[SO_2]^+$) group; a series of ions (*m/z* 79, 42%, *m/z* 80, 10%; *m/z* 81, 10%; *m/z* 82, 12%) was consistent with a piperidine ring resulting from thermal elimination of the two substituents and subsequent aromatization of the ring.

The ^{13}C NMR demonstrated the presence of the carboxyl group with a low field singlet (δ171.28). The structure including the chair conformation of PA1 was readily assigned by proton–proton shift correlated 2D NMR [5] as *trans*-4-hydroxypipecolic acid-4-sulphate (**1**). The Jeener and 500 MHz ^{1}H NMR of **1** are shown in Fig. 1. All the vicinal coupling constants between the proton on C-4 and the protons on C-3 and C-5 are small, thereby establishing the equatorial disposition of H-4. The chemical shift of H-4 (δ4.89) is significantly more deshielded than H-4 of free 4-hydroxypipecolic acid (δ4.2) [6] confirming the attachment of the sulphate group at C-4. The large $J_{2,3a}$ value (13.0 Hz) indicates that H-2 is axial, so that **1** exists in a chair conformation with an



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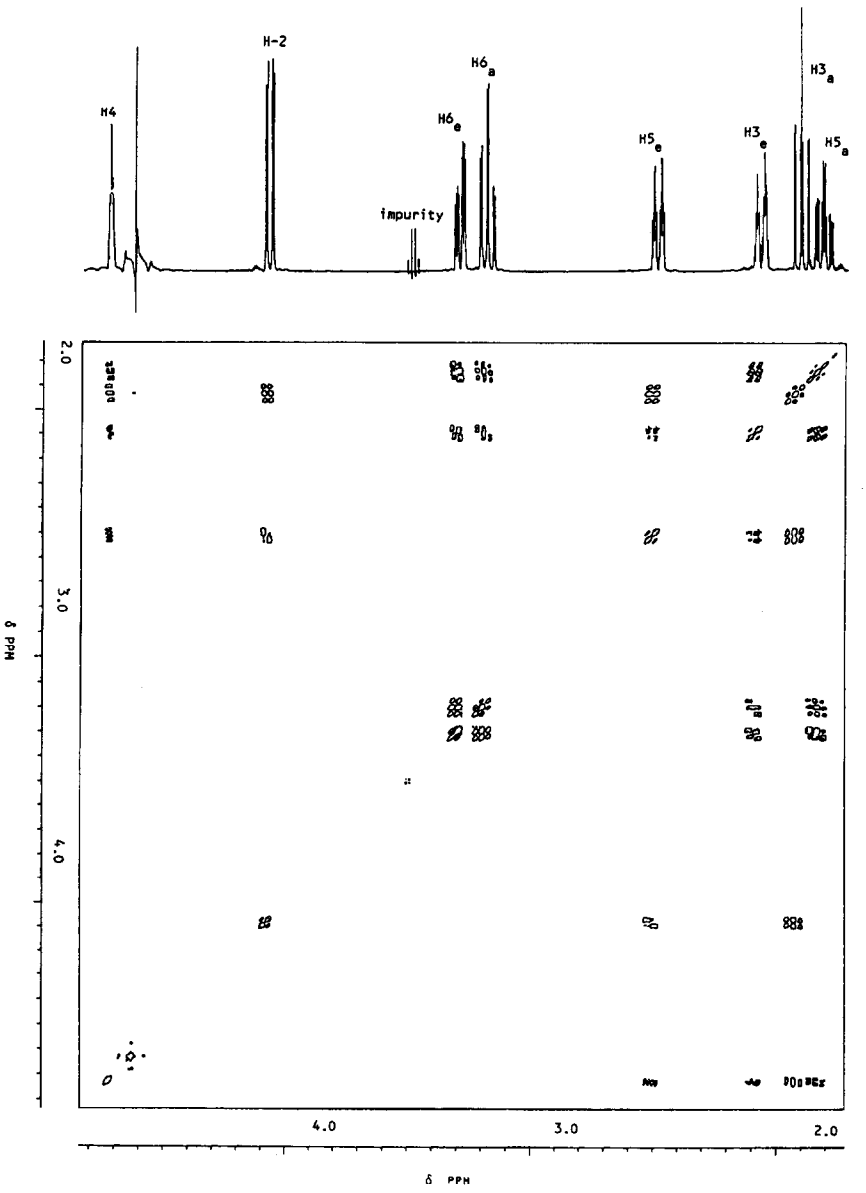

Fig. 1. 500 MHz NMR and Jeener spectrum of *trans*-4-hydroxypipecolic acid 4-sulphate in D_2O.

equatorial carboxyl group and an axial sulphate; *trans*-4-hydroxypipecolic acid has also been shown to exist in a chair conformation with an equatorial carboxyl group [6].

The accumulation of monohydroxy [3, 7, 8], dihydroxy [9], and trihydroxy [10] pipecolic acids in seeds of some species of Leguminosae is well documented. However, this is the first report of a conjugated monohydroxypipecolic acid or of a sulphate ester of a non-protein amino acid in plants.

The distribution of **1** in seed of species of *Peltophorum* and some allied genera is given in Table 1. With the exception of *P. dasyrrachis* Kurz ex Baker, all *Peltophorum* species are characterised by a high concentration of (**1**), whereas it is absent from species of *Campsiandra*, *Batesia* and *Vouacapoua*. This supplements an earlier report that the sulphate conjugate (when still uncharacterised) was also absent from other genera considered allied to *Peltophorum*, viz. *Bussea*, *Colvillea*, *Delonix* and *Schizolobium* [1]. The absence of (**1**) from *P. dasyrrachis* may prove taxonomically significant but it is not possible to make judgments regarding generic status from this data alone since morphological criteria for generic delineation within the group are not clearly defined. Taxonomic literature is scant and a revision of the genus possibly overdue.

Little is known of the ecological rôle or biological activity, if any, of the pipecolic acids. A recent report [11] states that pipecolic acid and various mono- and dihydroxy derivatives had an inhibitory effect on the

Table 1. Distribution of *t*-4-hydroxypipecolic acid 4-sulphate in seeds of *Peltophorum* and some allied genera

Genus/species	Source	Voucher	Zonal range and distribution	Concentration (1) in seed
CAMPSIANDRA Benth.				
C. comosa Benth.	Prance 21987, Oct. 1974, Brazil	K	S. America } (mainly Amazonia)	—
C. laurifolia Benth.	Silva 4288, Para, Brazil	SC	S. America }	—
BATESIA Spruce ex Benth.				
B. floribunda Spruce ex Benth.	Ducke 1146, 29.12.42, Manaus, Brazil	K	S. America (Amazonia)	—
VOUACAPOUA Aubl.				
V. americana Aubl.	No collectors data, Para, Brazil	SC	S. America (Amazonia)	—
PELTOPHORUM (Vogel) Benth.				
P. adnatum Griseb.	Fairchild Botanic Garden, Miami (cultivated)	SC	W. Indies	+ + +
P. africanum Sond.	Herbst s.n., 1975, S. Africa	SC	Southern tropical Africa	+ +
	Kirstenbosch Botanic Garden 1975 S. Africa	SC		+ +
	Robinson 247, 19.5.53, Mapanza Mission, Zambia	K		+ + +
	Hardy s.n., 1975, Australia	SC		+ + +
P. dasyrrachis Kurz ex Baker	Comanor 493, Peradeniya, Sri Lanka	K	Tropical Asia	—
	Larsen *et al.*, 31210, 1972, Krabi, Thailand	K		—
	Williams 19, 19.4.50, Tanganyika (cultivated)	K		—
P. dubium Taub. (= *P. vogelianum* Walp.)	Balansa 3081, April 1881, Mobatobi Plain, Paraguay	K	S. America (Brazil–N. Argentina)	+ +
	Montivideo Botanic Garden s.n., 1975, Uruguay	SC		+ +
P. ferrugineum Benth.	Dwyer 11960, Panama	SC	Australia–S. Asia	+ +
	Berry s.n., Aragua Botanic Garden, Maracay, Venezuela (cultivated)	SC		+ +
	Dept. of Forests s.n., Aug. 1974 New Guinea	SC		+ +
	Nicholson 48510, 27.2.65 Sandakhan, Sabah	K		+ +
P. inerme Naves ex Villar (= *P. ferrugineum* Benth.)	No collectors data, July 1974 Sri Lanka	SC	S. Asia	+ +
P. pterocarpum Backer ex Heyne	Singapore Botanic Garden s.n., 1975, Singapore.	SC	S. Asia	+ +
	Parnell 4056, 1.8.76, Maya Cove, Tortota, B. Virgin Islands.	K		+ +
	Deighton 5595, 19.9.51 Najala, Sierra Leone	K		+ +
P. tonkinense Gagnep.	Balansa 2183, May 1888, Tonkin China	K	S. E. Asia	+

+ + +, High ($\geq 0.5\%$ fr wt); + +, medium (0.1–0.5 % fr wt); +, low (0.02–0.1 % fr wt); t, trace (0.005–0.02 % fr wt); —, not detected. SC, Krukoff seed collection held at RBG Kew; K, Herbarium sheet, RBG Kew.

growth, metamorphosis and survival of *Spodoptera* larvae when incorporated into an artificial diet at levels of 0.1–5.0%. A preliminary study in this laboratory has shown that (1) has no effect on larval survival or adult emergence of the bruchid beetle *Callosobruchus maculatus* when incorporated at 1% into a cow pea flour diet [12]. This contrasts with the finding that many legume non-protein amino acids and alkaloids are toxic to this seed beetle when similarly incorporated at levels of 0.1–1.0% [12, 13].

EXPERIMENTAL

Isolation of trans-*4-hydroxypipecolic acid-4-sulphate* (*PA1*). Mature seed of *Peltophorum africanum* Sond. was collected in Kirstenbosch Botanic Garden, South Africa in 1975 and obtained for this study from the Krukoff Seed Collection held at RBG, Kew. Finely ground seed (65 g) was defatted with Me_2CO and extd with 2 × 500 ml 75% aq MeOH. The combined filtered MeOH exts were concd to 200 ml under red. pres. and applied to a column of Dowex 50-8 (35 × 2 cm, H^+ form). Washing with H_2O (250 ml) removed PA1 but all other amino acids remained on the resin. The fractions containing PA1 were pooled, evapd to dryness and the residue dissolved in H_2O (25 ml). This soln was applied to a DEAE cellulose column (Whatman DE 52, 20 × 2 cm). PA1 was displaced with 0.1 M HOAc (120 ml), evapd to dryness and recrystallised from hot H_2O to yield 276 mg, mp 248° (decomp); $[\alpha]_D^{20}$ + 6.5 (*ca* 0.2 in H_2O).

Analysis of extracts. Finely ground seed (200 mg) was shaken with 75% MeOH (1 ml) for 24 hr, filtered and the filtrate subjected to ionophoresis on Whatman No. 1 paper (70 v/cm for 30 min) at pH 3.6 [14]. Papers were then dried and developed with ninhydrin.

Structural analysis of PA1. Mp was recorded on a Kofler block. ^1H and ^{13}C NMR spectra were run at 500 MHz and 125 MHz, respectively, using D_2O as solvent (δ ppm from DSS). FAB-MS were run using Ar as reactant gas.

$C_6H_{11}N_1S_1O_6 \cdot H_2O$ (Found: C, 29.44; H, 5.40; N, 5.76; S, 12.49%. Calcd. C, 29.63; H, 5.35; N, 5.76; S, 13.17%). IR ν_{max}^{KBr} 1720, 1585, 1250, 855 cm^{-1}. ^1H NMR: δ 2.01, *dddd*, H-5*a*; 2.09, *ddd*, H-3*a*; 2.26, *dtt*, H-5*e*; 2.67, *ddt*, H-3*e*; 3.37, *dt*, H-6*a*; 3.47, *ddd*, H-6*e*; 4.24, *dd*, H-2; 4.89, *tt*, H-4. *J* (Hz) $J_{2,3a}$ 13.0; $J_{2,3e}$ 3.5; $J_{3a,3e}$ 15.4; $J_{3a,4}$ 2.4; $J_{3e,4}$ 3.5; $J_{4,5a}$ 2.4; $J_{4,5e}$ 3.5; $J_{5a,5e}$ 15.6; $J_{5a,6a}$ 13.2; $J_{5a,6e}$ 5.0; $J_{5e,6a}$ 3.5; $J_{5e,6e}$ 2.4; $J_{5e,3e}$ 2.4; $J_{6a,6e}$ 13.2 Hz. ^{13}C NMR: δ 171.28 (*s*, C-1), 70.17 (*d*, C-4), 52.19 (*d*, C-2), 38.69 (*t*), 30.18 (*t*), 26.07 (*t*).

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