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ALKALOIDS OF THE LEAVES OF *ERYTHROPHLEUM CHLOROSTACHYS*

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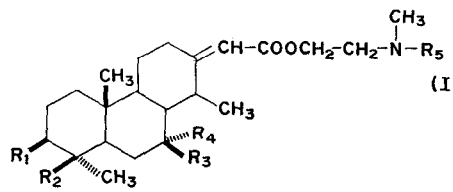
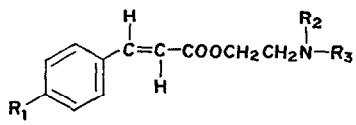
Abstract—From the leaves of *Erythrophleum chlorostachys* (F. Muell.) Bail. growing at Mareeba, North Queensland, β-dimethylaminoethyl cinnamate (II), *N*-2-hydroxyethyl-*N*-methyl cinnamamide (III), *N*-2-hydroxyethyl-*N*-methyl-*trans*-*p*-hydroxycinnamamide (IV) and *N*-2-hydroxyethylcinnamamide (V) were isolated. The structures were confirmed by synthesis. The amides (III), (IV) and (V) may possibly be artefacts of isolation since, as free bases, the cinnamic esters isomeric with (III) and (V) rearrange to (III) and (V) respectively, with half-lives less than 3 days and 1 day, respectively. Leaves of *E. chlorostachys* growing at Cooktown, North Queensland, and at Darwin, N.T., did not contain these compounds but contained alkaloidal esters of diterpenoid acids as in other *Erythrophleum* species.

INTRODUCTION

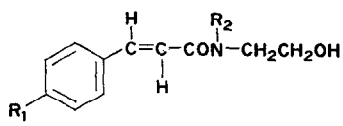
Erythrophleum chlorostachys (F. Muell.) Bail., Leguminosae, commonly known as Ironwood, is a large tree endemic in Northern Australia and distributed from the Kimberleys in Western Australia, through the Northern Territory and the islands of the Gulf of Carpentaria, to Northern Queensland. All parts of the tree are extremely poisonous and large numbers of sheep and cattle have died from eating the leaves.[1–3] Two leaves are reputed



(I) R_1=H, OH or O-acyl
R_2=CH_3 or CO_2CH_3
R_3R_4=H, OH or (=O)
R_5=H or CH_3



(II) R_1=H, R_2=CH_3, R_3=CH_3
(VI) R_1=H, R_2=CH_3, R_3=H
(VII) R_1=OH, R_2=CH_3, R_3=H
(VIII) R_1=H, R_2=H, R_3=H



(III) R_1=H, R_2=CH_3
(IV) R_1=OH, R_2=CH_3
(V) R_1=H, R_2=H

[1] L. J. WEBB, *Guide to the Medicinal and Poisonous Plants of Queensland*, C.S.I.R. Bull. 232 (1948).
[2] C. A. GARDNER and H. W. BENNETTS, *The Toxic Plants of Western Australia*, West Australian Newspapers, p. 37 (1956).
[3] *Poisonous Plants of the Northern Territory*, Northern Territory Administration Animal Industry Branch Extension Article No. 2, Part II (1957).

to be sufficient to kill a goat.[4] An early investigation by Petrie[5] disclosed no cyanogenetic glycoside or saponin but a small amount of alkaloid (0·002% in leaf, 0·03% in seed) with effects in the frog and dog similar to those of crude alkaloid of *E. guineense*, an ordeal tree of Central Africa. The alkaloid was amorphous and was not characterized. The alkaloid present in the leaves of *E. chlorostachys*, collected from localities near Mareeba, North Queensland, has now been re-examined.

From *E. guineense* and three other species, eight alkaloids have previously been isolated, all of which are esters of diterpene carboxylic acids with β-dimethylaminoethanol or β-methylaminoethanol.[6] Their structures are summarized by the general formula (I).

RESULTS AND DISCUSSION

The initial extraction of leaf from Mareeba gave crude alkaloid (0·5%) from which four substances were obtained in low yield by chromatography on alumina: *A*, $C_{13}H_{17}O_2N$, non-crystalline; *B*, $C_{12}H_{15}O_2N$, m.p. 77–78°; *C*, $C_{12}H_{15}O_3N$, m.p. 176–7°; and *D*, $C_{11}H_{13}O_2N$, m.p. 97°. Of these, only compound *A* was basic.

Compound A formed crystalline salts, the spectral properties of which indicated that the base was β-dimethylaminoethyl cinnamate (II). An IR spectrum of the oxalate showed peaks at 1640, 1720 (unsaturated ester) and 3420 cm^{-1} (NH). A mass spectrum of the picrate showed a molecular ion, *m/e* 219, and fragment ions, *m/e* 131, 103 and 77, appropriate for the partial structure Ph—CH=CH—CO. An NMR spectrum measured on approx. 2 mg picrate showed *N*-methyl and aromatic absorption and an indication of multiplets near δ 3·6 and 4·7 corresponding to the O—CH_2—CH_2—N^+ methylene groups. These indications were confirmed by direct comparison of the picrates of compound *A* and a synthetic sample of (II). Spectra of the oxalates were also identical.

The IR spectrum of compound *B* suggested an amide, the only carbonyl stretching absorption being at 1640 cm^{-1}. A strong peak at 3360 cm^{-1} indicated OH or NH. A strong molecular ion, *m/e* 205, in the mass spectrum confirmed the empirical formula and ions *m/e* 131, 103 and 77 again indicated Ph—CH=CH—CO. Other ions *m/e* 190 (M-CH_3), *m/e* 187 (M-H_2O), *m/e* 174 (M-CH_2OH), *m/e* 161 (M-CH_3CHO) and *m/e* 160 (M-CH_2CH_2OH) provided evidence for the grouping CO—N(CH_3)—CH_2—CH_2—OH. The NMR spectrum ($CDCl_3$, 40°) was of the poorly resolved type commonly observed for amides undergoing an interconversion of *cis*, *trans* isomers at a rate comparable with the time constant of the instrument. Nevertheless it contained peaks indicative of *trans* Ph—CH=CH—CO (large peak at δ 7·37 and an AB system, δ *c.* δ 6·9, 7·8, J 14·5 Hz), and multiplets (near δ 3·7 and 3·1) ascribable to O—CH_2—CH_2—NMe—CO. Thus it was possible to assign to *B* the structure *N*-2-hydroxyethyl-*N*-methylcinnamamide (III). This was confirmed by direct comparison with a synthetic sample.

Compound *C*, also non-basic, showed amide absorption at 1640 cm^{-1} and broad NH or OH stretching absorption near 3200 cm^{-1}. The mass spectrum showed a molecular ion, *m/e* 221, and fragment ions M-18, M-31, M-44 and M-45, indicative of CO—N—CH_2—CH_2—OH. The base peak, *m/e* 147, and a peak *m/e* 119 suggested that the cinnamic acid moiety of compounds *A* and *B* was, in this instance, replaced by C_6H_4(OH)—CH=CH—CO. NMR spectra definitely indicated that compound *C* was *N*-2-hydroxyethyl-*N*-methyl-*trans*-*p*-hydroxycinnamamide (IV) although an overlapping of peaks in the aromatic region

[4] F. M. BAILEY, *Queensland Agric. J.* **VII**, 153 (1900).
[5] J. M. PETRIE, *Proc. Linnean Soc. N.S.W.* **46**, 334 (1921); *Nature, Lond.* **108**, 231 (1921).
[6] R. B. MORIN, *The Alkaloids* (edited by R. H. F. MANSKE), Vol. 10, p. 287, Academic Press, New York (1968).

and the apparent occurrence of amide *cis, trans* isomerization made interpretation difficult. The aromatic signals were composed of a 4-proton AA'BB' quartet, δ 6·82, 7·46 (J 9·0 Hz) in deuteroacetone, partly obscured in deuteropyridine, and a 2-proton AB quartet, δ 7·23, 7·93 (J 15·0 Hz) in deuteropyridine, partly obscured in deuteroacetone. In deuteroacetone at 30°, the NMe signal appears as two broadened peaks, δ 2·98, 3·22, indicative of slow isomerization, and there is a multiplet centred near δ 3·6 ascribable to O—CH_2—CH_2—N. In deuteropyridine at 30°, the interconversion rate is faster and the NMe signal is a singlet δ 3·14, while the methylene groups give two approximate triplets δ 3·72, 3·95. At lower temperatures in deuteropyridine, the NMe peak splits into two. The structural assignment for compound *C* has also been confirmed by comparison with a synthetic sample of (IV).

Compound *D* showed amide carbonyl absorption at 1650 cm^{-1} and OH or NH absorption at 3300, 3370 cm^{-1}. The mass spectrum contained a molecular ion *m/e* 191, in agreement with the formula $C_{11}H_{13}O_2N$, and ions *m/e* 131 (base peak), 103 and 77 corresponding to Ph—CH=CH—CO. Additional ions, particularly *m/e* 173 (M-H_2O) and *m/e* 146 (M-CH_2CH_2OH) supported an *N*-CH_2CH_2OH grouping. The NMR spectrum also indicates a cinnamic acid moiety with a 5-proton multiplet, δ 7·3, and an AB quartet δ 6·46, 7·61 (J 15·0 Hz) the remaining signals constituting a 4-proton envelope centred at δ 3·7. The substance was therefore considered to be *N*-2-hydroxyethylcinnamamide (V), this being confirmed by comparison with a synthetic sample.

β-Dimethylaminoethylcinnamate (II) has been prepared previously from cinnamoyl chloride and β-dimethylaminoethanol,[7] and *N*-2-hydroxyethylcinnamamide (V) by heating ethyl cinnamate with β-aminoethanol.[8] The other amides (III) and (IV) were prepared by similar methods. The four substances were found to be identical with the isolates *A–D* from *E. chlorostachys* leaf.

Esters and amides of β-aminoethanol and β-methylaminoethanol are known to undergo interconversion readily, the esters generally being stable only in the protonated form.[9] At a pH above 9, they are said to rearrange to the amide. It was possible therefore that the amides (III), (IV) and (V) were artefacts of isolation and that the leaf constituents were the corresponding esters (VI), (VII) and (VIII). In order to ascertain the properties of these substances the esters (VI) and (VIII) have also been synthesized from the appropriate acid chloride and aminoethanol. The ester hydrochloride (VI.HCl) is found to be stable in neutral aqueous solution for at least a week. The free base of β-aminoethyl cinnamate (VIII) and β-methylaminoethyl cinnamate (VI) may be prepared from solutions of their hydrochlorides by treatment with sodium bicarbonate and extraction with chloroform. After two days at room temperature in deuterochloroform, β-aminoethyl cinnamate is entirely converted into amide as shown by NMR spectroscopy. On the other hand a little β-methylaminoethyl cinnamate is still present after 1 week, and its half-life is about 60 hr. When this ester is left in 20% NH_3 in ethanol for 2 months the main product is still the amide (III) (65%) rather than an ammonolysis product. The stability of *N*-2-hydroxyethyl-*N*-methyl-*trans*-cinnamamide (III) to acid was also examined. At room temperature in 10% hydrochloric acid substantial hydrolysis occurs over a week to give cinnamic acid, while in dry ethereal hydrogen chloride the amide is converted into the ester hydrochloride (VI.HCl) with a half-life of 65 hr as shown by NMR spectroscopy.

[7] G. Cerbai and G. F. Di Paco, *Boll. Chim. Farm.*, **105**, 45 (1966).
[8] O. K. Behrens, J. Corse, D. E. Huff, R. G. Jones, Q. F. Soper and C. W. Whitehead, *J. Biol. Chem.*, **175**, 771 (1948).
[9] T. C. Bruice and S. J. Benkovic, *Bioorganic Mechanisms*, p. 138, Benjamin Inc., New York (1966).

From these observations it is apparent that the esters (VI) and (VIII) and, by extension, (VII), would be converted into the corresponding amides (III), (V) and (IV) respectively, under the isolation conditions used. Thus it is possible that the esters occur naturally rather than the amides, a question which cannot be answered until more material can be obtained. Surprisingly, samples of dried leaf of *Erythrophleum chlorostachys* from Darwin and Cooktown appeared to contain only the diterpene ester alkaloids found in other *Erythrophleum* species. No signals due to cinnamates were detectable in NMR spectra of the crude alkaloid mixtures although signals were readily visible if 5% (w/w) of (V) were added to the crude alkaloid. A small amount of leaf was available from the original Mareeba collection,* having been stored under ethanol for approximately 3 yr. When extracted in such a way as to avoid isomerization, the alkaloid fraction from this material gave an NMR spectrum resembling that of the cinnamate (VIII), with no NMe peaks and no evidence of diterpene ester alkaloids. Only one species of *Erythrophleum* is recognized in Australia, but it seems that there are at least two chemical varieties sharply different in their alkaloidal constituents.

EXPERIMENTAL

Extraction of leaf alkaloids. The powdered leaf (5·8 kg) from Mareeba was moistened with 10% NH_4OH (2·4 l.), packed in a percolator immersed in an ice-bath, and extracted with Et_2O. The extract was concentrated and transferred to an ice-jacketed column of kieselguhr (350 g) moistened with 2 N HCl (100 ml). After pigments etc., were eluted with Et_2O, the column packing was extruded, made alkaline with ammonia and the alkaloids recovered with $CHCl_3$. The crude alkaloid (28·1 g) was separated into petrol-soluble, (14·2 g) benzene-soluble (5·4 g), Et_2O soluble (3·5 g) and $CHCl_3$-soluble (4·8 g) fractions, and each fraction was then separately chromatographed on alumina (activity III–IV).

Compound *A* was obtained from the petrol-soluble and benzene-soluble fractions as a gum by elution with benzene. With methanolic oxalic acid, it formed an oxalate, m.p. 186–187°. (Found: C, 58·6; H, 6·5; N, 4·9. Calc. for $C_{13}H_{17}O_2N.(CO_2H)_2$: C, 58·3; H, 6·2; N, 4·5%), and with ethereal picric acid a picrate, m.p. 178–179°. (Found: C, 50·7; H, 4·5; N, 12·3. Calc. for $C_{13}H_{17}O_2N.C_6H_3O_7N_3$: C, 50·9; H, 4·5; N, 12·5%.) Although these compounds could not be purified to constant m.p. because of insufficient material, they both gave IR spectra identical with those of the corresponding salts of β-dimethylaminoethyl cinnamate (II) described below. The mixed m.p. of the picrate was undepressed by a sample of the synthetic picrate and the mass spectra were the same.

Compound *B* was obtained from the petrol-soluble fraction by elution with ether and Et_2O–EtOH (4:1) and purified by preparative TLC, using layers of alumina G (Merck) and $CHCl_3$–EtOH (98:2) as solvent. It crystallized from Et_2O–petrol and had m.p. 77–78°. (Found: C, 70·1, H, 7·6; N, 6·8. Calc. for $C_{12}H_{15}O_2N$: C, 70·2; H, 7·4; N, 6·8%.) IR (KBr) 3380, 1640, 1590, 1110, 1025, 760 and 700 cm^{-1}. The m.p. was undepressed on admixture with an authentic sample of *N*-2-hydroxyethyl-*N*-methyl cinnamamide (III) prepared below and comparison of IR, NMR and mass spectra of both samples confirmed their identity.

Compound *C* was obtained from the Et_2O- and $CHCl_3$-soluble fractions by elution with $CHCl_3$ or EtOH. It crystallized from EtOH, m.p. 176–177°. (Found: C, 65·3; H, 6·9; N, 6·8. Calc. for $C_{12}H_{15}O_3N$: C, 65·1; H, 6·8; N, 6·4%.) IR (KBr) 3280 (br), 1640, 1600, 1580, 1510, 1180, 1065, 1040, 830, 810 cm^{-1}. The m.p. was undepressed on admixture with an authentic sample of *N*-2-hydroxyethyl-*N*-methyl-*trans*-*p*-hydroxycinnamamide (IV) prepared below, and comparison of IR, NMR and mass spectra of both samples confirmed their identity.

Compound *D* was obtained from the Et_2O soluble material by elution with Et_2O–EtOH (4:1) and from the $CHCl_3$-solubles by elution with $CHCl_3$. It was purified by preparative TLC and crystallized from $CHCl_3$-light petroleum or EtOH-light petroleum, m.p. 97°. (Found: C, 68·9; H, 6·9; N, 7·2. Calc. for $C_{11}H_{13}O_2N$; C, 69·1; H, 6·9; N, 7·3%.) IR (KBr) 3450–3250 (br), 3070, 1650, 1600, 1555, 1450, 1340, 1080, 1050, 1020, 760, 710 cm^{-1}. The m.p. was undepressed on admixture with an authentic sample of *N*-2-hydroxyethylcinnamamide (V) prepared below and comparison of IR, NMR and mass spectra of both samples confirmed their identity.

SYNTHETIC COMPOUNDS

Preparation of Amides

A general method was used in which a methyl or ethyl ester and an excess of the β-aminoethanol were heated for 18 hr at 120°. The excess amine was removed by washing with dilute acid, and the product purified by crystallization.

* Herbarium specimen, HN.VKM 557, stored at C.S.I.R.O., Long Pocket Laboratories, Indooroopilly, Queensland.

N-2-Hydroxyethyl-*trans*-cinnamamide (V), obtained in 40% yield, crystallized from benzene, m.p. 101° (recorded[8] m.p. 101°).

N-2-Hydroxyethyl-*N*-methyl-*trans*-cinnamamide (III) was obtained in 69% yield. The *product* crystallized from benzene, m.p. 78°, λ_{max} (EtOH) 217, 222, 278 nm (ε 16,700, 13,700, 22,900). (Found: C, 70·4; H, 7·6; N, 6·5. $C_{12}H_{15}NO_2$ required: C, 70·2; H, 7·4; N, 6·8%.)

N-2-Hydroxyethyl-*N*-methyl-*p*-hydroxy-*trans*-cinnamamide (IV) was obtained in 50% yield. The *product* crystallized from MeOH, m.p. 177°, λ_{max} (EtOH) 212, 227, 299, 312 nm (ε 11,200, 13,400, 22,800, 24,700). (Found: C, 65·0; H, 6·7; N, 6·3. $C_{12}H_{15}NO_3$ required: C, 65·1; H, 6·8; N, 6·3%.)

Preparation of esters

2-*Aminoethyl* trans-*cinnamate* (*VIII*). A mixture of β-aminoethanol hydrochloride (4·9 g) and cinnamoyl chloride (10 g) were dissolved in diethylene glycol diethyl ether (25 ml) by heating and left overnight. The crystals (3·8 g) which separated were filtered off and washed with $CHCl_3$ and Et_2O and crystallized from MeOH–Et_2O, followed by isopropanol. 2-Aminoethyl *trans*-cinnamate hydrochloride formed needles, m.p. 182°, ν_{max} (KBr) 1720, 1650 cm^{-1} (—CH=CH—CO) (Found: C, 58·0; H, 6·2; N, 6·1. $C_{11}H_{14}ClNO_2$ required C, 58·0; H, 6·2; N, 6·2%.) NMR spectrum (Varian A60 spectrometer) in $(CD_3)_2SO$ solution showed signals at δ 3·16 (t, J 5 Hz, 2H, NCH_2—); 4·38 (t, J 5 Hz, 2H, OCH_2—); 6.60 (d, J 16 Hz, 1H, C=CHCO); 7·3–7·85 (m, 5H, ArH); 7·90 (d, J 16 Hz, 1H, C=CH-Ar); 8·4 (broad m, 3H, N^+H_3).

The *free base* was recovered as an oil by neutralizing an aqueous solution of the hydrochloride with $NaHCO_3$, extracting with $CHCl_3$, and evaporating the $CHCl_3$ below 45°. NMR ($CDCl_3$ solution): δ 1·42 (broad m, 2H, NH_2); 3·00 (broad m, 2H, CH_2N); 4·23 (t, J 5·5 Hz, 2H, OCH_2); 6·45 (d, J 16, 1H, C=CHCO); 7·3–7·7 (m, 5H, ArH); 7·72 (d, J 16, 1H, ArCH=C). The NMR solution was re-run after standing for 2 days, and the spectrum then corresponded to that of the amide.

2-*N-Methylaminoethyl* trans-*cinnamate* (VI). β-Methylaminoethanol (3·75 g) was treated with excess dry HCl in EtOH-free $CHCl_3$ (200 ml) to give the hydrochloride in the form of a second liquid phase. Cinnamoyl chloride (10 g) was added, the mixture warmed on a steam bath and set aside for 2 days by which time it was a homogeneous solution. The $CHCl_3$ was evaporated and the residue twice crystallized from EtOH-free $CHCl_3$ to give the *hydrochloride* (6·2 g), needles m.p. 162°, ν_{max} ($CHCl_3$) 1715, 1640 cm^{-1} (—CH=CH—CO—). (Found: C, 59·4; H, 6·7; N, 5·6. $C_{12}H_{16}ClNO_2$ required: C, 59·6; H, 6·8; N, 5·8%.) The NMR spectrum ($CDCl_3$ solution) showed signals at δ 2·76 (broad s, 3H, NMe); 3·30 (broad s, 2H, —CH_2N); 4·58 (t, J 5·0 Hz, 2H, —CH_2O); 6·31 (d, J 16 Hz, 1H, C=CHCO); 7·2–7·7 (m, 5H, ArH); 7·86 (d, J 16 Hz, 1H, ArCH=C).

The free base, isolated as described previously, was an oil, ν_{max} ($CHCl_3$) 1700, 1640 cm^{-1} (—CH=CH—CO), with an NMR spectrum ($CDCl_3$ solution) showing signals at δ 2·04 (s, NH); 2·47 (s, NMe); 2·88 (unresolved t, —CH_2N); 4·30 (t, J 5·5 Hz, —CH_2O); 6·44 (d, J 16 Hz, C=CHCO), 7·2–7·6 (m, ArH); 7·69 (d, J 16 Hz, ArCH=C) and signals in the region δ 3–4 from amide impurity amounting to 15 per cent calculated by comparison of the integration curve for the NMe groups. After 1 week the spectrum was re-run and showed 86% amide.

β-Dimethylaminoethyl cinnamate[7] gave a *hydrobromide*, m.p. 130° (EtOH). (Found: C, 51·9; H, 6·0; N, 5·0; Br, 26·2. $C_{13}H_{17}NO_2$. HBr required: C, 52·0; H, 6·0; N, 4·7; Br, 26·6%.) The *picrate* formed yellow micro-crystals from acetone, m.p. 183–5°. (Found: C, 50·7; H, 4·6; N, 12·7. $C_{19}H_{20}O_9N_4$ required: C, 50·9; H, 4·5; N, 12·5%.) The *oxalate* formed needles from methanol, m.p. 191·5°. (Found: C, 57·9; H, 6·3; N, 4·5. $C_{13}H_{17}NO_2.C_2H_2O_4$ required: C, 58·2; H, 6·2; N, 4·5%.)

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