s, pyronic hydrogen), 4·75 (2H, *m*, 2 C=CH–), 6·55 (4H, *d*, *J* 8 Hz, 2 $-CH_2-Ar$), 7·66 (3H, *s*, pyronic methyl), 8·18 (6H, *s*, 2 Me–C≡) and 8·25 (6H, *s*, 2 Me–C≡). This compound was synthesized by Seshadri *et al.* [6], mp 149–150°, identical NMR spectrum. The other compounds isolated were identified by comparison with authentic samples.

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N-METHYL-L-TYROSINE FROM SEEDS OF *COMBRETUM ZEYHERI**

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Early reports of the isolation of a nitrogenous compound from the bark of the legumes *Geoffroya surinamensis*, *Ferreira spectabilis* and *Andira anthelmintica* and an American ratanhia extract were summarized by Johnson and Nicolet [2]. The compound was named by various workers, surinamine, geoffroyine, ratanhine, angeline and andirine before the identity of the different isolates was recognized. Goldschmiedt [3] confirmed earlier work which indicated that the empirical formula of the compound was $C_{10}H_{13}NO_3$ and established that his "ratanhine" gave a negative optical rotation in acid solution. Subsequent syntheses of racemic *N*-methyltyrosine [4] and of the D and L isomers [5, 6] confirmed that the natural compound was N-methyltyrosine and that the specific rotation reported by Goldschmiedt for the natural product corresponded to that of the synthetic D isomer.

While investigating the free amino acids present in the seeds of an East African plant, *Combretum zeyheri* (Combretaceae), we observed several ninhydrin-reacting "spots" occupying unfamiliar positions on 2D paper chromatograms of the seed extract. One of the more prominent of these gave a brown-purple reaction with ninhydrin and moved slightly faster than tyrosine in both solvents. The compound was isolated by ion exchange chromatography and characterized as *N*-methyl-L-tyrosine, identical in all respects to a synthetic sample.

EXPERIMENTAL

Paper chromatography. Finely ground seed (100 mg) was shaken with 70% EtOH (1 ml) for 2 hr at room temp. After standing for a further 17 hr the suspension was centrifuged and the supernatant liquid used for analyses. 2D chromatograms were descending on Whatman 3 MM paper using 0·1 ml of extract. Solvents used were (1) *n*-BuOH–HOAc–H_2O

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(90:10:29) [7] and (2) PhOH–H_2O (4:1, w/v) in the presence of the vapour of aq. NH_3 (sp. gr. 0·88).

Ionophoresis. Ionophoresis was conducted on Whatman 3 MM paper (70 V/cm for 30 min) in buffer solutions of pH 1·9, pH 3·6 and pH 6·5 [8].

Colour reactions. With ninhydrin the amino acid gave a brown–purple; it showed no chelation with Cu^{2+}, indicating no unsubstituted α-amino group [9], it gave orange with *p*-nitrobenzoyl chloride indicating an *N*-methyl-α-amino group [10]. It gave orange with Pauly's reagent suggesting a phenol [11].

Isolation of N-*methyl*-L-*tyrosine.* Finely ground seed (2 kg) was steeped in 75% EtOH (20 l.) for 2 weeks with occasional shaking. After filtering, the residue was reextracted with 20 l. 75% EtOH for 1 week. The combined extracts were concentrated to 8 l. and passed through a column (120 × 4·5 cm) of strongly acidic ion-exchange resin (Dowex 50 × 8) in the H^+ form. The column was washed with 15 l. H_2O. The last 10 l. of washings gave a +ve ninhydrin reaction and were passed through a second column (32 × 4·8 cm) of Amberlite IR 120 in the H^+ form. The washings (10 l. H_2O) gave no reaction with ninhydrin. The amino acids were displaced with 2 N NH_3 and the solns combined. A ppt. which formed was filtered and recrystallized from H_2O to give a colourless solid (1·2 g). The filtrate was evaporated to dryness and the "crystalline" residue (320 g) and the solid mixed and stirred with hot H_2O (4 l.) but did not dissolve completely; the insoluble material was filtered, washed (H_2O 500 ml) and dried (5·1 g). The washings and filtrate were combined (4·5 l.) and *iso*-PrOH (225 ml) added. The soln was passed through a column (77 × 3·8 cm) of ion-exchange resin (Dowex 50 × 8) in the NH_4^+ form. The column was then washed with H_2O (15 l.). The effluent and washings (containing acidic and neutral amino acids) were combined and evaporated under red. pres. to 4 l. The pH was adjusted to 4·8 (HOAc) and the soln passed through a column (88 × 3·8 cm) of Dowex 50 × 5 in the H^+ form. The column was washed (H_2O, 10 l.) and the amino acids displaced with NH_3 (0·25 M). The effluent fractions were analysed by high voltage electrophoresis and PC, and the fractions containing the amino acid which moved close to tyrosine on the 2D chromatograms were combined (2·4 l.) and concentrated to 400 ml. The soln was decolourized (C, 50 g). The charcoal was washed with H_2O (3 l.) and the conc washings (25 ml) added to the decolourized soln. After standing at 6° for 17 hr a crop of colourless crystals was obtained (5 g). These crystals and the 5·1 g of material which failed to dissolve in the 4·5 l. of hot H_2O were found (by electrophoresis at pH 1·9) to be the "unknown" contaminated with traces of tyrosine. The tyrosine was removed by recrystallization from H_2O and the purified amino acid obtained in colourless needles. $[\alpha]_{23}^{D} = +16{\cdot}0$ (*ca* 0·42; M HCl); $[\alpha]_{23}^{D} = +31{\cdot}45$ (*ca* 0·42; M NaOH).

UV spectrum λ_{max} 275 and 223 nm in 0·1 M HCl and at 292 and 239 nm in 0·1 M NaOH. [Cf tyrosine [12] at 274·5 and 223 nm and 293 and 240 nm respectively.]

Circular dichroism. The isolated compound gave a doubly positive Cotton effect [$\Delta\epsilon + 2{\cdot}5$, λ_{max} (224 nm); $\Delta\epsilon + 2{\cdot}5$, λ_{208} nm] cf L-tyrosine [$\Delta\epsilon + 2{\cdot}36$ at λ_{max} (227 nm); $\Delta\epsilon + 2{\cdot}13$, λ_{205} nm].

NMR spectrum. ^{13}C spectra were determined with solutions of the amino acid in 2 N NaOD/D_2O using a Bruker HFX-90 spectrometer operating in Fourier Transform mode at 22·628 MHz with proton broad-band decoupling. ^1H spectra were obtained from the same solutions operating the spectrometer at 90·000 MHz. The ^{13}C spectrum, referred to external TMS, showed 8 single peaks when broad-band decoupled from ^1H. Off centre decoupling indicated the nature of one-bond ^{13}CH coupling in the molecule. There was close similarity to the spectrum of L-tyrosine except for the presence of one extra peak which had a quartet structure when off-centre decoupled, the aromatic signals showed that the compound was para substituted and the chemical shifts were consistent with –O– rather than –OMe substitution. The following assignments were made: C_1 = 183·3 ppm (*s*), C_2 = 68·5 (*d*), C_3 = 39·4 (*t*); phenyl ring [13] $C_{1'}$ = 125·1 (*s*), $C_{2',6'}$ = 131·8 (*d*), $C_{3',5'}$ = 119·9 (*d*), $C_{4'}$ = 165·8 (*s*) and C_{Me} = 34·6 (*q*). The PMR spectrum, referred to DDS, showed signals at δ 2·20 (3H, *s*), 4·91 (*s*, exchangeable protons), a second order region centred at 2·7 (2H) and 3·1 (1H) and a second order AB pattern centred at 6·5 (2H) and 6·9 (2H). Multiplication of the free induction decay by an exponential function [exp 3·0] gave a significant improvement in resolution and the aromatic region of the spectrum was seen as a simple AA′ BB′ system, whilst the second order aliphatic region was an AB_2 system. The spectra were simulated to within the limits of experimental error on a Nicolet 1083 computer using the following parameters. AA′ BB′ system: δ 6·96 (A), 6·58 (B), $J_{AB} = J_{A'B'}$ 8·8 Hz, $J_{AA'}$ 2·0 Hz, $J_{BB'}$ 1·0 Hz. $J_{AB'} = J_{B'A}$ 0·5 Hz. AB_2 system: δ 3·18 (A), 2·71 (B) J_{AB} 7·5 Hz.

The aliphatic region showed chemical shifts similar to those of L-tyrosine except for the presence of an extra methyl group, and the aromatic region was also similar to that of L-tyrosine. The chemical shift of the methyl group which showed no coupling to the rest of the molecule was closer to that of an *N*-methyl than to that of an *O*-methyl group and the chemical shift of the aromatic signals suggested the presence of an –O– group in basic solution rather than an –OMe group. The PMR spectrum was therefore consistent with that expected for *N*-methyltyrosine.

N-*methyl*-L-*tyrosine* was synthesized by the method of Corti [6]. The UV, NMR, IR and MS of the synthetic compound were identical to those of the isolated material. $[\alpha]_{23}^{D} = +16{\cdot}3$ (*ca* 0·276; MHCl); $[\alpha]_{23}^{D} = +29{\cdot}10$ (*ca* 0·423; M NaOH). The natural compound and the synthetic gave the same colour reactions and R_f values on paper and migrated together on electrophoresis at pH 1·9, 3·6 and 6·5.

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