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ISOLATION AND SYNTHESIS OF *TRANS*- AND *CIS*-(−)-CLOVAMIDES AND THEIR DEOXY ANALOGUES FROM THE BARK OF *DALBERGIA MELANOXYLON*

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Key Word Index—*Dalbergia melanoxylon*; bark metabolites; (−)-*trans*- and (−)-*cis*-clovamides; deoxyclovamides; synthesis; L-DOPA; 3-(4-hydroxyphenyl)-L-alanine; *N*-cinnamoyl derivatives; L-proline; ^{13}C and CD spectra.

Abstract—*N*-(3′,4′-Dihydroxy-*trans*-cinnamoyl)-3-(3,4-dihydroxyphenyl)-L-alanine [(−)-clovamide], the major phenolic metabolite (0.1%) in the bark of *Dalbergia melanoxylon*, is associated with minor proportions of its *cis*-isomer, and similar pairs of geometrical isomers of their deoxy analogues *N*-(4′-hydroxycinnamoyl)-3-(3,4-dihydroxyphenyl)-L-alanine and *N*-(4′-hydroxycinnamoyl)-3-(4-hydroxyphenyl)-L-alanine. (−)-*Trans*-clovamide is synthesized by direct condensation of the acid chloride of caffeic acid with L-DOPA. Diagnostic CD spectra of these compounds and ^{13}C spectra of (−)-*trans*- and (−)-*cis*-clovamides are recorded.

INTRODUCTION

The tree *Dalbergia melanoxylon* Guill. & Perr., commonly known as zebra wood, ebony, or African blackwood, has a wide distribution in Africa ranging from northern Zululand in the south, through northern Transvaal, Mozambique, Botswana and Zimbabwe-Rhodesia to tropical Africa and Ethiopia [1]. Its smooth, light grey bark is shown to contain *N*-cinnamoyl derivatives of amino acids in addition to free imino acids. The former group of compounds is of both medicinal and biosynthetic interest.

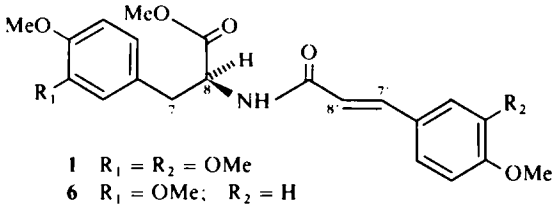
RESULTS AND DISCUSSION

Proof of structure of the main metabolite, *N*-(3′,4′-dihydroxy-*trans*-cinnamoyl)-3-(3,4-dihydroxyphenyl)-L-alanine (*trans*-clovamide [2,3]) is demonstrated as previously indicated [2,3] by its hydrolysis to 3,4-dihydroxyphenyl-L-alanine (**5**), commonly known as L-DOPA. The 3,4-dihydroxy-*trans*-cinnamoyl residue could not be traced after such hydrolysis, but its presence is demonstrated by successive methylation of the parent compound to the tetra-*O*-methyl ether methyl ester (**1**); hydrogenation of the cinnamoyl moiety to the dihydrocinnamoyl methyl ether derivative (**3**); and by hydrolysis of the latter to 3,4-dimethoxydihydrocinnamic acid (**4**). Photolysis of the methylated *trans*-clovamide (**1**, $J_{\alpha,\beta}$ = 15.0 Hz) at 350 nm in MeOH gives the corresponding *cis*-isomer (**2**, $J_{\alpha,\beta}$ = 12.5 Hz), the latter geometrical isomer being identical to a derivative of an associated minor metabolite in the bark. The structures of the full methyl ether esters of *trans*- and *cis*-clovamides (**1**) are confirmed by ^{13}C NMR spectroscopy, the more significant differences being the upfield position of the β-carbon resonances of the *trans*- relative to the *cis*-isomer (δ 141.2, 134.9 respectively, Δδ 6.3) and the reverse (δ 117.5, 121.2 respectively, Δδ −3.8) for α-carbon resonances (cf. Table 1).

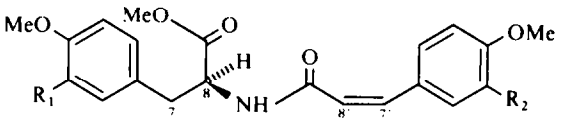
(−)-*Trans*-clovamide and its *cis*-isomer occur in the bark and in the solid extractives (MeOH) in 0.35 and 10.0%, and 0.04 and 1.32% concentrations, respectively. Both *trans*- and *cis*-isomers are known compounds, hitherto exclusive to the leaves and stems of the red clover (*Trifolium pratense*), and termed clovamides by Sakamura *et al.* [2,3]. However, no previous attempt at synthesis of the *trans*-clovamide or of its derivatives has been recorded. Synthesis of the free phenolic and optically pure form poses the problem of the possible racemization of the amino acid (L-DOPA) in the presence of base. This is avoided by direct condensation of the acid chloride of caffeic acid (*trans*-3,4-dihydroxycinnamoyl chloride) with 3-(3,4-dihydroxyphenyl)-L-alanine to give the *trans*-clovamide in 32% yield. However, synthesis of the acid chloride of caffeic acid by a method (using thionyl chloride) generally applicable to cinnamic acids [4] gives erratic results, and presently represents a limiting factor in the direct synthesis.

Circular dichroism of the natural and synthetic *trans*-clovamides shows, from the reduced amplitudes of the Cotton effects of the former (cf. Fig. 1), that the natural product is partly racemized. Comparing the CD spectra of derivatives of *trans*- and *cis*-clovamides and their deoxy analogues also permits distinction between these groups of geometric isomers (Figs. 2 and 3), the sign of the short and long wavelength Cotton effects (−, + and +, + respectively) being in each instance in line with calculated and observed spectra for chiral *trans*- and *cis*-α,β-unsaturated ketones [5].

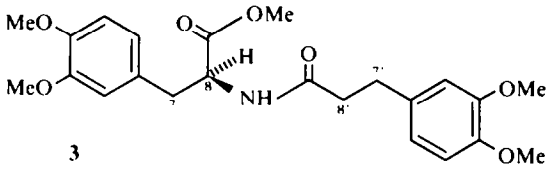
Novel deoxy analogues of the clovamides represent minor metabolites in the bark of *D. melanoxylon*, namely the pairs of *trans* and *cis* geometric isomers of *N*-(4′-hydroxycinnamoyl)-3-(3,4-dihydroxyphenyl)-L-alanine (both derivatives of L-DOPA; identified as their methyl ether esters, **6** and **7**), and of *N*-(4′-hydroxycinnamoyl)-3-(4-hydroxyphenyl)-L-alanine (identified as methyl ether esters, **8** and **9**). All are individually present in the bark and solid extractives at *ca* 0.0007 and 0.02% levels, respectively.



1 $R_1 = R_2 = OMe$
6 $R_1 = OMe$; $R_2 = H$
8 $R_1 = R_2 = H$



2 $R_1 = R_2 = OMe$
7 $R_1 = OMe$; $R_2 = H$
9 $R_1 = R_2 = H$



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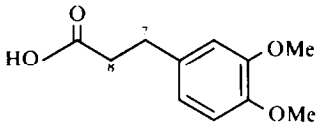

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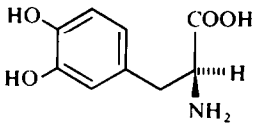

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Their CD spectra are similar to those of the natural and synthetic clovamide derivatives (Figs. 2 and 3), and the parent compounds accordingly possess the same absolute configurations at the chiral centres of their respective amino acid moieties.

Significant concentrations of sucrose (0.23, 6.57% in the bark and extractives respectively), the imino acid L-proline (0.11, 3.28%), and an unidentified imino acid (0.06, 1.71%) are also present.

The clovamides and their analogues are speculatively considered as 'trapped' precursors in view of the complete absence of associated flavonoids. By contrast, isoflavans [6], and isoflavone and rotenoid glycosides [7] characterize the bark of closely-related *D. nitidula*. The function of the clovamides and associated analogues in the bark of *D. melanoxylon* may be protective, as illustrated by inhibition of anthracnose, one of the main diseases of red clover (*Trifolium pratense*), by the former [3].

The main component, *trans*-clovamide, being constituted of both L-DOPA and caffeoyl moieties, may be of medicinal interest considering that caffeoyl derivatives of dihydroxyphenylalkanol heterosides enhance the therapeutic value of L-DOPA in Parkinsonism by inhibiting extracerebral DOPA decarboxylase [8] (cf. ref. [9]).

EXPERIMENTAL

NMR spectra were recorded on a Bruker WP-80 spectrometer for solutions in $CDCl_3$ (Me_4Si as internal reference) and ^{13}C allocations made on the basis of Sford multiplicities. UV spectra

Table 1. ^{13}C NMR chemical shifts for (−)-*trans*- and (−)-*cis*-clovamides

Allocation*	δ(CDCl$_3$) (−)-*Trans*-clovamide	(−)-*Cis*-clovamide	Δδ
C-9	171.6	168.8	2.8
C-9′	165.1	163.5	1.6
C-3, C-4	150.2, 148.9	147.3, 146.7	
C-3′, C-4′	148.6, 147.6	146.1, 145.5	
C-7′	141.2	134.9	6.3
C-1, C-1′	128.1, 127.2	126.2, 125.6	
C-6, C-6′	121.6, 120.9	119.4, 119.4	
C-8′	117.5	121.2	−3.8
C-5, C-5′	112.2, 110.9	111.3, 111.3	
C-2, C-2′	110.8, 109.4	109.4, 108.8	
4 × OMe	55.6	55.3	0.3
C-8	53.2	52.8	0.4
C-10	51.6	51.6	0
C-7	37.5	37.3	0.2

* No differentiation is possible within the various group allocations.

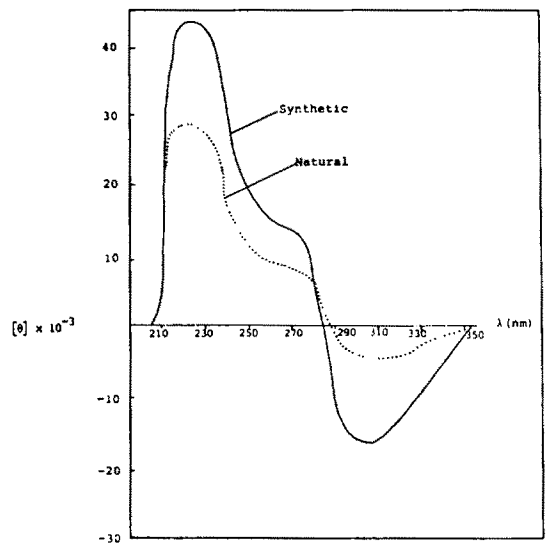

Fig. 1. CD spectra of synthetic and natural (−)-*trans*-clovamide.

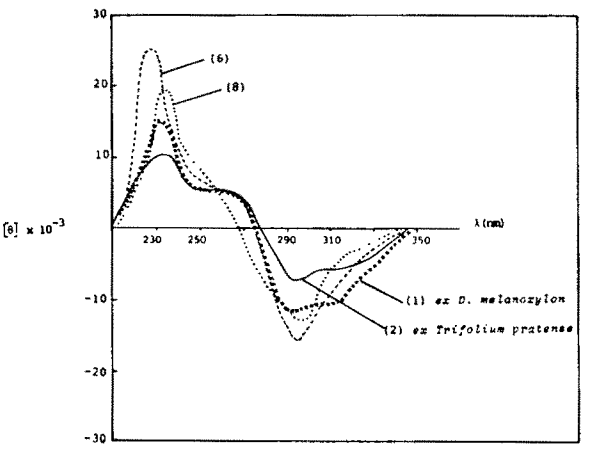

Fig. 2. CD spectra of the methyl ethers of (−)-*trans*-clovamide (**1**) and of its deoxy analogues, **6** and **8**.

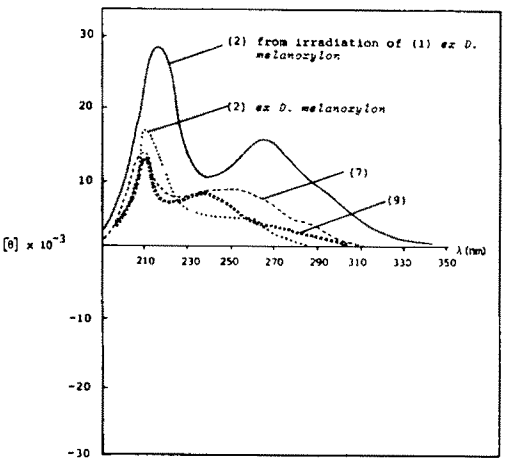

Fig. 3. CD spectra of the methyl ethers of (−)-*cis*-clovamide (**2**) and of its deoxy analogues, **7** and **9**.

were in MeOH. MS were obtained with a Varian CH-5 instrument. A JASCO J-20 spectropolarimeter was employed for CD determinations using MeOH as solvent. Media used for separation of components comprised Whatman No. 3 paper (preparative paper chromatography), Merck Si gel 60 (column chromatography), and Merck Si gel 60 PF_{254} (prep. TLC). TLC bands were located under UV illumination and/or with $FeCl_3/HClO_4$ spray reagent.

Extraction and preliminary separation. The bark (pulverized and dried, 2 kg) of *D. melanoxylon* was successively extracted with EtOAc (4 × 2 l., 24 hr each) and MeOH (5 × 2 l., 24 hr each). Evapn of the EtOAc extract produced a brown oil (8.5 g), which was chromatographed on a column (C_6H_6–hexane–EtOAc, 5:4:1) to yield 5 crude fractions. NMR investigation of these

fractions showed them to consist of mixtures of long-chain aliphatic substances only, which were not further investigated. The MeOH extract produced a brown solid (70 g) on evapn of the solvent. Prep. PC (2% HOAc; upward migration) of a portion (30 g) yielded 3 crude fractions (A, R_f 0.5, 5 g; B, R_f 0.6, 500 mg; C, R_f 0.9, 6 g). Methylation of a portion (500 mg) of fraction A and the subsequent separation of the methyl ethers by TLC (hexane–Me_2CO, 65:35) produced 2 compounds (**1**, **2**--R_f 0.20 and 0.26 respectively).

Pentamethyl-trans-*clovamide* (**1**). Solids from the band, R_f 0.20, crystallized from MeOH as white needles, which were identified as pentamethyl-*trans*-clovamide, mp 153°. Mmp and lit. [3] 152–153°. Found: M^+, 429.181; $C_{23}H_{27}NO_7$ requires: 429.179; MS *m/e* (rel. int.): 429 (41, M^+), 223 (57), 222 (100), 207 (67), 206 (92), 191 (75), 176 (23), 163 (50), 151 (83); UV λ_{max} (log ε): 315 (4.25), 285 (4.25), 230 (4.31), 205 (4.33); CD: (*c*, 0.0594) $[\theta]_{350}$ 0, $[\theta]_{290}$ − 12 272, $[\theta]_{274}$ 0, $[\theta]_{260}$ 5060, $[\theta]_{230}$ 12 727, $[\theta]_{205}$ 0; ^{1}H NMR: δ 7.47 (*d*, J = 15 Hz, H-7'), 7.06–6.44 (*m*, H-2, 5, 6, 2', 5', 6'), 6.19 (*d*, J = 15 Hz, H-8'), 6.02 (*d*, J = 5 Hz, NH), 4.91 (*m*, J = 5 and 7.5 Hz, H-8), 3.81 (*s*, 2 × Ar–OCH_3), 3.75, 3.73 (*s*, 2 × Ar–OCH_3), 3.69 (*s*, CO_2CH_3), 3.09 (*d*, J = 5 Hz, H-7).

Pentamethyl-cis-*clovamide* (**2**). Pentamethyl-*cis*-clovamide was isolated from the band, R_f 0.26, as a colourless oil. Found: M^+, 429.184; $C_{23}H_{27}NO_7$ requires: 429.179; MS *m/e* (rel. int.): 429 (6, M^+), 223 (16), 222 (100), 207 (49), 206 (73), 191 (51), 163 (7), 151 (71); UV λ_{max} (log ε): 312 (4.35), 283 (4.39), 230 (4.52), 205 (4.61); CD: (*c*, 0.0748) $[\theta]_{310}$ 0, $[\theta]_{270}$ 4000, $[\theta]_{235}$ 1233, $[\theta]_{210}$ 0; ^{1}H NMR: δ 7.23 (*d*, J = 2.5 Hz, H-2'), 6.98 (*dd*, J = 2, 5 and 8 Hz, H-6'), 6.69 (*d*, J = 8 Hz, H-5'), 6.64 (*d*, J = 7.5 Hz, H-5), 6.61 (*d*, J = 12.5 Hz, H-7'), 6.45 (*d*, J = 2 Hz, H-2), 6.41 (*dd*, J = 2 and 7.5 Hz, H-6), 5.95 (*d*, J = 7.5 Hz, NH), 5.73 (*d*, J = 12.5 Hz, H-8'), 4.83 (*m*, J = 5 and 7.5 Hz, H-8), 3.81 (*s*, 2 × Ar–OCH_3), 3.78, 3.72 (*s*, 2 × Ar–OCH_3), 3.64 (*s*, CO_2CH_3), 3.00 (*d*, J = 5 Hz, H-7).

Photolysis of pentamethyl-trans-*clovamide*. Pentamethyl-*trans*-clovamide (60 mg) in MeOH (60 ml) was irradiated for 6 hr (N_2 atmosphere, 350 nm). Evapn of the solvent and TLC separation (hexane–Me_2CO, 65:35) yielded a product (R_f 0.26, 27 mg) identical to pentamethyl-*cis*-clovamide (**2**).

Pentamethyldihydro-trans-*clovamide* (**3**). Pentamethyl-*trans*-clovamide (50 mg) in EtOH (50 ml) was hydrogenated (room temp., 1 hr) over 10% Pd–C. After filtration and evapn of the solvent, the product was crystallized from MeOH to yield pentamethyldihydro-*trans*-clovamide (45 mg) as white needles, mp 103–104°. Mmp and lit. [3] 103°. MS *m/e* (rel. int.): 431 (41, M^+), 224 (10), 223 (42), 222 (86), 210 (40), 209 (68), 207 (24), 192 (10), 191 (37), 180 (38), 165 (28), 164 (42), 152 (42), 151 (100), 150 (10), 149 (21), 147 (11), 137 (12), 135 (19), 121 (14), 108 (21), 107 (40), 106 (23); ^{1}H NMR: δ 6.88 6.34 (*m*, H-2, 5, 6, 2', 5', 6'), 5.78 (*d*, J = 7.5 Hz, NH), 4.83 (*m*, J = 5 and 7.5 Hz, H-8), 3.81 (*s*, 3 × Ar–OCH_3), 3.78 (*s*, Ar–OCH_3), 3.69 (*s*, CO_2CH_3), 3.00 (*d*, J = 5 Hz, H-7), 2.84 (*m*, H-7'), 2.47 (*m*, H-8').

3,4-*Di*-O-*methyldihydrocaffeic acid* (**4**). Pentamethyldihydro-*trans*-clovamide (70 mg) was refluxed with 6 N HCl (12 ml) for 6 hr and the product extracted with Et_2O (3 × 50 ml) following dilution with H_2O. Evapn and TLC separation (C_6H_6–Me_2CO, 8:2) yielded 3,4-di-*O*-methyldihydrocaffeic acid (R_f 0.26, 10 mg) which crystallized from MeOH as white needles, mp 95–97°. Lit. [3] 96.5–97.5°, MS *m/e* (rel. int.): 210 (99, M^+), 195 (32), 165 (33), 164 (32), 151 (100), 137 (28), 136 (20), 135 (35), 121 (39); ^{1}H NMR: δ 6.75 (*m*, Ar–H), 3.84, 3.81 (*s*, 2 × OCH_3), 2.92 (*m*, H-7), 2.64 (*m*, H-8); identical to the synthetic product obtained by successive methylation of caffeic acid and hydrogenation and hydrolysis of the ester.

L-*DOPA* (**5**). A second portion of fraction A (100 mg) was hydrolysed by refluxing with 6 N HCl (12 ml, 6 hr). After extraction with Et_2O (3 × 50 ml) the aq. layer was evapd to dryness. Prep. PC (MeOH–H_2O–10 N HCl–pyridine, 73:16:2:9) produced a component (R_f 0.6) which was identical to commercial L-DOPA, mp 283°. Lit. [10] 282°. MS *m/e* (rel. int.): 197 (35, M^+), 152 (27), 123 (100); CD: (*c*, 0.06 in 0.1 N HCl) $[\theta]_{300}$ 0, $[\theta]_{250}$ 163, $[\theta]_{210}$ 5727, $[\theta]_{200}$ 0. Fraction B was chromatographed on a column ($CHCl_3$–Me_2CO–MeOH–H_2O, 40:40:15:5) to yield 2 subfractions (B_1, R_f 0.33, 40 mg; B_2, R_f 0.28, 45 mg). Subsequent methylation of these with CH_2N_2 followed by TLC separation (*n*-hexane–Me_2CO, 65:35) produced 2 compounds (**6**, **7**–R_f 0.48 and 0.43, respectively) from B_1 and two (**8**, **9**—R_f 0.36 and 0.30, respectively) from B_2.

Methyl ester of N-(4'-*methoxy*-cis-*cinnamoyl*)-3-(4-*methoxyphenyl*)-L-*alanine* (**9**). The compound with R_f 0.48 (from B_1) was isolated as a white amorphous solid (6 mg), mp 89°. Found: M^+, 369.158; $C_{21}H_{23}NO_5$ requires: 369.158; MS *m/e* (rel. int.): 369 (8.2, M^+), 193 (15), 192 (99), 177 (75), 176 (97), 161 (100), 133 (30), 121 (98); UV λ_{max} (log ε): 275 (4.47), 205 (4.59); CD: (*c*, 0.075) $[\theta]_{350}$ 0, $[\theta]_{290}$ 2454, $[\theta]_{255}$ 7363, $[\theta]_{240}$ 6151, $[\theta]_{230}$ 12 303, $[\theta]_{215}$ 0; ^{1}H NMR δ 7.81 (*d*, J = 8.5 Hz, H-2', 6'), 6.94–6.70 (*m*, 6 × Ar-H, H-7'), 5.89 (*d*, J = 7.5 Hz, NH), 5.73 (*d*, J = 12.5 Hz, H-8'), 4.84 (*m*, J = 5 and 7.5 Hz, H-8), 3.75, 3.72 (*s*, 2 × OCH_3), 3.66 (*s*, CO_2CH_3), 2.89 (*d*, J = 5 Hz, H-7).

Methyl ester of N-(4'-*methoxy*-trans-*cinnamoyl*)-3-(4-*methoxyphenyl*)-L-*alanine* (**8**). The second compound (R_f 0.43) from the methylated fraction of B_1 was isolated as a white amorphous solid (7 mg), mp 127–129°. Found: M^+, 369.158; $C_{21}H_{23}NO_5$ requires: 369.158; MS *m/e* (rel. int.): 369 (11, M^+), 193 (17), 192 (99), 177 (78), 176 (99), 162 (19), 161 (100), 133 (33), 121 (98); UV λ_{max} (log ε): 288 (4.57), 205 (4.65); CD: (*c*, 0.061) $[\theta]_{330}$ 0, $[\theta]_{295}$ − 13 030, $[\theta]_{267}$ 0, $[\theta]_{235}$ 19 696, $[\theta]_{210}$ 0; ^{1}H NMR: δ 7.50 (*d*, J = 15 Hz, H-7'), 7.36 (*d*, J = 7.5 Hz, H-2', 6'), 6.95 (*d*, J = 8 Hz, H-2, 6), 6.79 (*d*, J = 7.5 Hz, H-3', 5'), 6.73 (*d*, J = 8 Hz, H-3, 5), 6.17 (*d*, J = 15 Hz, H-8'), 5.90 (*d*, J = 7.5 Hz, NH), 4.92 (*m*, J = 5 and 7.5 Hz, H-8), 3.78, 3.71 (*s*, 2 × Ar-OCH_3), 3.68 (*s*, CO_2CH_3), 3.12 (*d*, J = 5 Hz, H-7).

Methyl ester of N-(4'-*methoxy*-cis-*cinnamoyl*)-3-(3,4-*dimethoxy-phenyl*)-L-*alanine* (**7**). The compound with R_f 0.36 was isolated from the methylated reaction of B_2 as a colourless oil (6 mg). Found: M^+, 399.170; $C_{22}H_{25}NO_6$ requires: 399.168; MS *m/e* (rel. int.): 399 (5, M^+), 223 (18), 222 (100), 207 (16), 206 (23), 191 (20), 161 (39), 151 (54); UV λ_{max} (log ε): 284 (4.41), 207 (4.57), CD: (*c*, 0.07) $[\theta]_{330}$ 0, $[\theta]_{270}$ 7696, $[\theta]_{245}$ 6848, $[\theta]_{230}$ 13 393, $[\theta]_{210}$ 0; ^{1}H NMR: δ 7.43 (*d*, J = 8 Hz, H-2', 6'), 6.87–6.38 (*m*, 5 × Ar-H, H-7'), 5.91 (*d*, J = 7.5 Hz, NH), 5.72 (*d*, J = 12.5 Hz, H-8'), 4.84 (*m*, J = 7.5 and 5 Hz, H-8), 3.78, 3.74, 3.72 (*s*, 3 × Ar-OCH_3), 3.64 (*s*, CO_2CH_3), 2.98 (*s*, J = 5 Hz, H-7).

Methyl ester of N-(4'-*methoxy*-trans-*cinnamoyl*)-3-(3,4-*dimethoxyphenyl*)-L-*alanine* (**6**). The second component from the methylated fraction of B_2 (R_f 0.30) was isolated as an oil (6 mg). Found: M^+, 399.168; $C_{22}H_{25}NO_6$ requires: 399.168; MS *m/e* rel. int.): 399 (7, M^+), 223 (20), 222 (100), 207 (30), 206 (39), 191 (28), 161 (47), 151 (69); UV λ_{max} (log ε): 305 (4.38), 285 (4.40), 225 (4.44), 205 (4.47); CD: (*c*, 0.0748) $[\theta]_{330}$ 0, $[\theta]_{295}$ − 16 100, $[\theta]_{273}$ 0, $[\theta]_{250}$ 6700, $[\theta]_{225}$ 24 500, $[\theta]_{210}$ 0; ^{1}H NMR: δ 7.49 (*d*, J = 15 Hz, H-7'), 7.34 (*d*, J = 8 Hz, H-2', 6'), 6.97–6.50 (*m*, 5 × Ar-H), 6.10 (*d*, J = 15 Hz, H-8'), 5.93 (*d*, J = 7.5 Hz, NH), 4.93 (*m*, J = 7.5 and 6 Hz, H-8), 3.78, 3.76, 3.75 (*s*, 3 × Ar-OCH_3), 3.69 (*s*, CO_2CH_3), 3.12 (*d*, J = 6 Hz, H-7).

Synthesis of trans-clovamide. L-DOPA (20 mg) and caffeoyl chloride (40 mg) in dry THF were stirred for 48 hr at room temp. (cf. ref. [4]). The unreacted L-DOPA was filtered off and the solvent evapd. Column chromatography ($CHCl_3$–Me_2CO–MeOH–H_2O, 40:40:15:5) yielded *trans*-clovamide (10 mg, R_f 0.18) as an amorphous solid, mp 125–130°. CD: (*c*, 0.0888) $[\theta]_{350}$ 0, $[\theta]_{300}$ − 1618, $[\theta]_{285}$ 0, $[\theta]_{275}$ 1454, $[\theta]_{225}$ 4448, $[\theta]_{205}$ 0; by comparison, values for the natural product are

$[\theta]_{350}$ 0, $[\theta]_{300}$ −480, $[\theta]_{285}$ 0, $[\theta]_{275}$ 850, $[\theta]_{225}$ 2730, $[\theta]_{205}$ 0.

Penta-O-methyl-trans-clovamide. Methylation of the prepared *trans*-clovamide with dimethyl sulphate and crystallization from MeOH produced the pentamethyl ether as white needles (6 mg), mp 153°. Lit. [3] 152–153°. The mass and NMR spectra were identical with those of the methyl ether of the natural product.

Carbohydrate and imino acid fraction. Fraction C (1 g) was rechromatographed (PC; EtOAc–pyridine H_2O, 2:2:1, upward migration) into 2 subfractions (C_1 and C_2).

Sucrose. Fraction C_1 (R_f 0.53, 400 mg) consisted of a single substance which showed the same R_f value (EtOAc–pyridine–H_2O, 2:2:1) colour reaction with aniline–diphenylamine–phosphoric acid spray reagent [11] as a sucrose reference sample. Octa-*O*-acetylsucrose (10 mg) was obtained by refluxing sucrose (15 mg) with Ac_2O–pyridine for 8 hr. The product was crystallized from EtOH to yield white needles, mp 71°. Lit. [12] 72.3°. ^{1}H NMR: δ 5.63–4.63 (*m*, 3 × CH_2), 4.38–4.00 (*m*, 8 × CH), 2.11 (*s*, 2 × OAc), 2.08 (*s*, 2 × OAc), 2.10 (*s*, 2 × OAc), 2.00, 1.98 (*s*, 2 × OAc). Fraction C_2 (R_f 0.15, 300 mg) was separated by column chromatography ($CHCl_3$–Me_2CO–MeOH–H_2O, 2:2:4:2) into 2 components, R_f 0.46, 0.56.

L-*Proline.* The substance with the lower R_f (0.46), which developed a yellow colour with ninhydrin spray reagent [11], was identified as L-proline (18 mg), ^{1}H NMR: δ(D_2O) 5.60–5.88 (1 H, *m*, H-2), 6.32–6.60 (2 H, *m*, H-5), 7.32–7.95 (4 H, *m*, H-3,4); MS *m/e* (rel. int.) 115 (10.4, M^+), 70 (100). The substance with R_f 0.56 (30 mg) developed a pink colour with ninhydrin spray reagent. ^{1}H NMR: δ(D_2O) 5.43–6.00 (2 H, *m*), 6.38–6.66 (1 H, *m*), 7.00–7.75 (2 H, *m*); MS *m/e* (rel. int.): 145 (16, M^+), 100 (100). The ^{1}H NMR spectrum of this substance is closely related to that of 4-hydroxyproline and the substance appears to be an analogue containing an 'extra' methylene function.

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REFERENCES

1. Palmer, E. and Pittman, N. (1972) *The Trees of Southern Africa*, Vol. II, p. 935. Balkema, Cape Town.
2. Yoshihara, T., Yoshikawa, H., Sakamura, S. and Sakuma, T. (1974) *Agric. Biol. Chem. (Jpn)* **38**, 1107.
3. Yoshihara, T., Yoshikawa, H., Kunimatsu, S., Sakamura, S. and Sakuma, T. (1977) *Agric. Biol. Chem. (Jpn)* **41**, 1679.
4. Womack, E. B. and McWhirter, J. (1955) *Organic Synthesis*. (Horning, E. C., ed.) Collective Vol. III, p. 714. Wiley, New York.
5. Hug, W. and Wagnière, G. (1971) *Helv. Chim. Acta* **54**, 633.
6. Van Heerden, F. R., Brandt, E. V. and Roux, D. G. (1978) *J. Chem. Soc. Perkin Trans.* **1**, 137.
7. Van Heerden, F. R., Brandt, E. V. and Roux, D. G. (1980) *J. Chem. Soc. Perkin Trans.* **1**, (in press).
8. Societe Civile de Recherches et d'Etudes Nouvelles, Ger. Offen. 2,609,533 (16 September 1976) (cf. (1977) *Chem. Abstr.* **86**, 60518k).
9. Hartman, W. J., Akawie, R. I. and Clark, W. G. (1955) *J. Biol. Chem.* **216**, 507.
10. Bell, E. A., Nulu, J. R. and Cone, C. (1971) *Phytochemistry* **10**, 2191.
11. Stahl, E. (1969) *Thin-Layer Chromatography*, pp. 856, 889. Springer, Berlin.
12. Hassid, W. Z., Doudoroff, M. and Barker, H. A. (1944) *J. Am. Chem. Soc.* **66**, 1416.