Pergamon

0031-9422(95)00452-1

Phytochemistry, Vol. 40, No. 5, pp. 1563–1565, 1995

0031-9422/95 $9.50 + 0.00

2,7-DIHYDROXY-3-FORMYL-1-(3′-METHYL-2′-BUTENYL)CARBAZOLE FROM *CLAUSENA LANSIUM*

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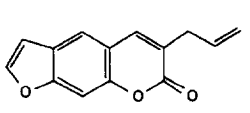
(*Received in revised form* 22 *March* 1995)

Key Word Index—*Clausena lansium*; Rutaceae; root bark; coumarins; carbazole alkaloids; 2,7-dihydroxy-3-formyl-1-(3′-methyl-2′-butenyl)carbazole.

Abstract—*Clausena lansium* root bark contained chalepensin, chalepin, gravelliferone, angustifoline, indizoline and the new carbazole alkaloid, 2,7-dihydroxy-3-formyl-1-(3′-methyl-2′-butenyl)carbazole.

INTRODUCTION

Clausena lansium is a shrub which has been introduced into Sri Lanka. Its roots and fruits are utilized in Chinese folk medicine [1], while its leaves are used in Sri Lanka as a substitute for curry leaf in cooking. Previous studies on *C. lansium* include the isolation of carbazole alkaloids from the root [1] and leaf [2], and a secondary amine from the leaf [2]. We now report the presence of four coumarins, chalepensin, chalepin, gravelliferone and angustifolin, together with sitosterol and the carbazole alkaloids, indizoline and the new 2,7-dihydroxy-3-formyl-1-(3′-methyl-2′-butenyl)carbazole.



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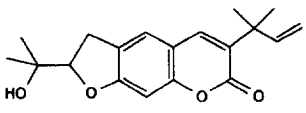

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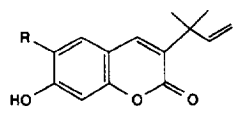

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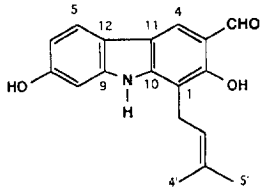

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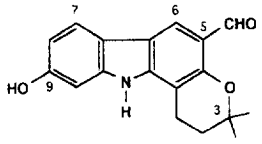

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RESULTS AND DISCUSSION

The dichloromethane extract of *C. lansium* root bark contained the 3-(1′,1′-dimethylallyl) coumarins, chalepensin (**1**), chalepin (**2**), gravelliferone (**3**) and angustifolin (**4**), together with sitosterol and two carbazole alkaloids, indizoline and a new carbazole, 2,7-dihydroxy-3-formyl-1-(3′-methyl-2′-butenyl)carbazole (**5**). The ^{13}C NMR data of **1**–**4** which have not been previously reported are given in Table 1.

Carbazole **5** gave a yellow colour with Dragendorff's reagent and its UV spectrum was characteristic of carbazole alkaloids. The IR spectrum of **5**, molecular formula $C_{18}H_{17}O_3N$ from HR mass spectrometry, showed a broad band at ν_{max} 3500–2400 cm^{-1} for chelated OH and NH groups and an intense peak at ν_{max} 1660–1610 cm^{-1} for a carbonyl group.

The ^{1}H NMR spectrum of **5** contained two methyl singlets, a CH_2 doublet and a CH triplet characteristic of an *iso*pentenyl substituent on an aromatic ring. A singlet at δ9.80 indicated the presence of an aldehyde group, while molecular formula considerations suggested that two hydroxyl substituents were also present. Four aromatic proton signals in its ^{1}H NMR spectrum gave additional evidence that **5** was a tetrasubstituted carbazole.

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Table 1. ^{13}C NMR assignments of the 3-(1′,1′-dimethyl-allyl)coumarins **1**–**4**

Carbon	1	2	3	4
2	159.9	162.3	161.1	161.6
3	133.2	131.0	134.7	130.9
4	146.6†	145.7	145.6	145.3
5	119.5	123.3	128.1	129.1
6	124.6	124.6	131.0	112.3
7	155.9*	160.2	157.5*	159.6
8	99.0	97.2	102.5	102.6
9	151.3*	154.7	153.2*	154.7
10	115.9	113.2	112.7	112.8
1′	40.5	40.4	40.3	40.3
2′	138.3	138.1	138.6	138.9
3′	112.3	112.1	112.1	113.7
1′-Me	26.2, 26.2	26.1, 26.2	26.2, 26.2	26.2, 26.2
1″	106.3	29.7	28.4	—
2″	145.5†	91.0	121.3	—
3″	—	71.8	125.4	—
3″-Me	—	24.3, 24.3	17.9, 25.8	—

*† Assignments interchangeable.

The singlet at δ7.86 indicated that three aromatic substituents were on the A ring. The remaining aromatic signals consisting of a double doublet (J = 8.4 and 2.1 Hz) and two doublets, with J = 8.4 and 2.1 Hz, respectively, suggested that these protons were in the C ring and had a 1,2,4-relationship with each other. The fourth substituent must therefore be at C-6 or C-7. The ^{1}H–^{1}H HOMOCOSY spectrum of **5** gave support to these conclusions.

The mass spectrum of the alkaloid showed intense peaks at m/z 240 [M–CH=CMe$_2$]$^+$ and 211 [240–CHO]$^+$ giving further evidence for the presence of *iso*pentenyl and aldehyde groups.

The NOESY spectrum of **5** showed correlations between the CHO proton and the singlet at δ7.86 indicating that the CHO group was *ortho*- to the unsubstituted position in the A ring. The absence of correlations between these protons and the side-chain CH$_2$ group suggested that the latter was not in close proximity to either of them. Unambiguous information on NOESY correlations of the NH proton is not available because CD$_3$OD had to be used to dissolve **5** for NMR.

The HMQC spectrum permitted the assignment of the non-quaternary carbon atoms, while the HMBC spectrum allowed the positions of the substituents to be unequivocally determined. HMBC correlations between the side-chain methyl protons and the carbon signals at δ121.5 and 133.8 allowed them to be assigned to C-2′ and C-3′, respectively. The side-chain CH$_2$ showed correlations to these two signals and also to the quaternary carbon atoms at δ109.6, 145.8 and 157.3. The signal at δ157.3 which should be due to a carbon carrying an OH substituent was also correlated with the CHO proton and the A ring proton signals, while the signal at δ145.8 was correlated only with the A ring proton signal in the HMBC. The side-chain could therefore only be at C-1 (δ109.6) with the OH and CHO groups, respectively, at C-2 and C-3, leaving C-4 as the unsubstituted position in the A ring. The signal at δ145.8 must then be due to C-10.

The lone substituent in C-ring must be an OH group. The carbon atom carrying this group (δ156.3) showed correlations with the doublet at δ7.72, which was also correlated with the quaternary carbon signals at δ142.7 (C-9) and 118.3. The 4-H signal was correlated with the signal at δ116.9, which showed correlations with the C-ring proton at δ6.84. The signals at δ118.3 and 116.9 were therefore assigned to C-11 and C-12, respectively. The OH substituent in C-ring must be at C-7 and the proton at δ6.84 should be due to H-8.

The carbazole **5** must therefore have the structure, 2,7-dihydroxy-3-formyl-1-(3′-methyl-2′-butenyl)carbazole. While H-5 had the expected NOESY relationship with H-6, that with H-4 was not clear due to the close proximity of the H-4 signal. However, none was also observed between H-4 and the doublet at δ6.84 as would be expected if the OH group was at the 6-rather than the 7-position. Evidence for the proposed structure also came from its UV spectrum which was very similar to that of its 7-methyl ether, 7-methoxyheptaphylline [3].

Formic acid cyclization of **5** gave an isomeric compound **6**, whose ^{1}H and ^{13}C NMR spectra were similar to those of **5**. A six-proton singlet at δ1.43 and two mutually coupled CH$_2$ triplets in the ^{1}H NMR spectrum indicated that the side-chain had cyclized to give a dimethylpyran ring. The CHO and H-4 singlets were both shifted downfield by $\sim\delta$0.5 probably due to stereochemical changes on cyclization. In the ^{13}C NMR spectrum of **6**, the pyran ring carbons appeared as two CH$_2$ signals at δ26.8 and 31.7 and a quaternary signal at δ75.1. The side-chain unsaturated carbon signals at δ120.3 and 133.5 were no longer present, while the C-1, C-3, C-4 and CHO signals were shifted slightly. The product of cyclization was therefore 3,3-dimethylpyrano-[5-6*a*]-5-formyl-9-hydroxy-1*H*,11*H*-carbazole (**6**) and its formation was further evidence for the structure proposed for **5**.

EXPERIMENTAL

General. UV: MeOH. IR: KBr. MS: EI 70 eV. ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR: CDCl$_3$ (CDCl$_3$ + 2 drops CD$_3$OD for **5** and **6**), TMS int. standard, correlations from ^{1}H–^{1}H-HOMOCOSY; ^{13}C NMR assignments from APT, HMQC and HMBC. Optical rotations: CDCl$_3$, 22°. Medium pressure liquid (MPLC) and flash (FC) chromatography: Merck Kieselgel 9385. Identities of compounds were established by mmp, IR and ^{1}H NMR comparisons unless otherwise stated. *Clausena lansium* root bark was collected from Deltota, in central Sri Lanka, in January 1993 and a voucher specimen has been deposited in the University Herbarium.

Extraction. Air-dried root bark (1.25 kg) was extracted at 27° with CH$_2$Cl$_2$ for two 24 hr periods each. Concentration gave the CH$_2$Cl$_2$ extract (27.3 kg).

Chromatography and identification. The extract (27 g) was slurried with silica gel (27 g) and subjected to MPLC

on silica gel (105 g). Elution with hexane–CH_2Cl_2 (3:2) gave a fr., which recrystallized from hexane–CH_2Cl_2 as colourless needles of chalepensin (**1**) (1.25 g), mp 89–90° (lit. [4] mp 87–88°); MS *m/z* (rel. int.): 254 $[M]^+$ (100), 239 (94), 225 (19), 211 (72), 199 (96), 171 (27), 155 (46), while elution with hexane–CH_2Cl_2 (1:1) gave a fr. which on FC with hexane–EtOAc (3:2) yielded indizoline (94 mg), yellow plates from hexane–CH_2Cl_2, mp 167–168° (lit. [1] mp 169–170°) and gravelliferone (**3**) (30 mg), colourless needles from hexane–CH_2Cl_2, mp 166–167° (lit. [5] mp 166–168°); MS *m/z* (rel. int., %): 298 $[M]^+$ (46), 283 (70), 255 (30), 243 (100), 227 (29), 215 (25) 199 (27) and elution with hexane–CH_2Cl_2 (2:3) gave sitosterol, mp 138° (lit. [4] mp 138°). The above compounds were found to be identical to authentic materials.

Elution with CH_2Cl_2–MeOH (97:3) gave chalepin (**2**) as brownish-yellow beads (1.64 g) from EtOAc–hexane, mp 119–120°, $[\alpha]_D = +43.7°$ (*c* 0.2) (lit. [4] mp 118–119°, $[\alpha]_D$ + 28°) (HRMS: 314.1526 $[M]^+$, Calc. for $C_{19}H_{22}O_4$: 314.1518); MS *m/z* (rel. int.): 314 $[M]^+$ (39), 299 (37), 281 (94), 255 (30), 255 (25), 241 (11), 199 (24) and 59 (100) and with EtOAc–hexane (1:1), angustifolin (**4**), colourless plates from hexane–CH_2Cl_2 (1.12 g), mp 136–137° (lit. [6] gum) (HRMS: 230.0937 $[M]^+$, Calc. for $C_{14}H_{14}O_3$: 230.0943); MS *m/z* (rel. int.): 230 $[M]^+$ (77), 215 (100), 187 (79), 175 (92), 147 (21), 115 (29), 51 (49), whose UV, IR and ^1H NMR spectral data were identical with those reported.

Elution with CH_2Cl_2–MeOH (24:1) gave a fr. which on FC (CH_2Cl_2–MeOH, 24:1) gave a yellow solid which recrystallized from CH_2Cl_2–MeOH as yellow needles of 2,7-dihydroxy-3-formyl-1-(3′-methyl-2′-butenyl)carbazole (**5**) (48 mg), mp 194–196° (HRMS: 295.1208 $[M]^+$, Calc. for $C_{18}H_{17}O_3N$: 295.1203); UV $\lambda_{max.}$ nm (log ε): 340 (4.05), 302 (4.71) and 238 (4.43), IR $\nu_{max.}$ cm^{-1}: 3500–2400, 1660–1610, 1300, 1200 and 1140; ^1H NMR (500 MHz): δ1.68 (3H, *d*, *J* = 1 Hz, H-5′), 1.83 (3H, *s*, H-4′), 3.55 (2H, *d*, *J* = 6.8 Hz, CH_2), 5.28 (1H, *br t*, *J* = 6.8 Hz, = CH), 6.72 (1H, *dd*, *J* = 8.4 and 2.1 Hz, H-6), 6.84 1H, *d*, *J* = 2.1 Hz, H-8), 7.72 (1H, *d*, *J* = 8.4 Hz, H-5), δ7.86 (*s*, H-4), 9.80 (1H, *s*, CHO); ^{13}C NMR (125 MHz): δ18.2 (C-4′), 23.2 (C-1′), 25.9 (C-5′), 97.7 (C-8), 109.6 (C-1), 109.7 (C-6), 115.2 (C-3), 116.9 (C-12), 118.3 (C-11), 120.7 (C-5), 121.5 (C-2′), 124.2 (C-4), 133.8 (C-3′), 142.7 (C-9), 145.8 (C-10), 156.3 (C-7), 157.3 (C-2), 195.8 (CHO); MS m/z (rel. int.): 295 $[M]^+$ (67), 280 (23), 240 (100), 211 (23), 183 (20), 154 (15), 77 (8).

Cyclization of **5**. **5** (20 mg) was stirred with HCO_2H for 3 hr at 24°. The blue soln was dild (H_2O), extracted with CH_2Cl_2, washed (aq. $NaHCO_3$), dried (Na_2SO_4) and concd to give an oil (20 mg) which on prep. TLC (2 × CH_2Cl_2) gave 3,3-dimethylpyrano-[5-6*a*]-5-formyl-9-hydroxy-1*H*,11*H*-carbazole (**6**) (12 mg), mp 211–213° (HRMS: 295.1208 $[M]^+$, Calc. for $C_{18}H_{17}NO_3$: 295.1203); ^1H NMR δ1.43 (6H, *s*, 3-Me), 1.97 (2H, *t*, *J* = 6.8 Hz, H-2), 2.86 (2H, *t*, *J* = 6.8 Hz, H-1), 6.74 (1H, *dd*, *J* = 8.4 and 2.2 Hz, H-8), 6.85 (1H, *d*, *J* = 2.2 Hz, H-10), 7.75 (1H, *d*, *J* = 8.4 Hz, H-7), 8.26 (1H, *s*, H-6), 10.40 (1H, *s*, CHO); ^{13}C NMR: δ 17.8 (CH_3), 26.8 (C-1), 29.7 (CH_3), 31.7 (C-2), 75.1 (C-3), 97.3 (C-10), 102.5 (C-1a), 109.3 (C-8), 112.4 (C-5), 116.8 (C-7a), 117.3 (C-6a), 117.7 (C-6), 120.7 (C-7), 142.1 (C-10a), 144.5 (C-11a), 155.0 (C-4a), 155.5 (C-9), 190.6 (CHO); MS *m/z* (rel. int.): 296 $[M + 1]^+$ (70), 295 (56), 241 (100), 212 (17), 184 (15), 155 (12), 55 (7).

Acknowledgements—The authors thank Mr Siril Wijesundera of the Royal Botanic Gardens, Peradeniya, for identifying the plant material. Thanks to Dr Ulla Jacobsson of the Royal Institute of Technology, Stockholm for assisting us with 500 MHz NMR data. Thanks also to the International Program in Chemical Sciences, Uppsala, Sweden, for financial support and Deutscher Akademischer Austauschdienst (DAAD), Bonn for fellowships to V.K. and A.C.A.

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