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CLAUSENOL AND CLAUSENINE—TWO CARBAZOLE ALKALOIDS FROM *CLAUSENA ANISATA*

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Key Word Index—*Clausena anisata*; Rutaceae; stem bark; clausenol; clausenine; carbazole alkaloids; antimicrobial activity.

Abstract—Two new carbazole alkaloids, designated as clausenol and clausenine, were isolated from an alcoholic extract of the stem bark of *Clausena anisata*. Their structures were established as 1-hydroxy-6-methoxy-3-methylcarbazole and 1,6-dimethoxy-3-methyl carbazole, respectively, from physical and chemical evidence and synthesis. Clausenol was found to be active against Gram-positive and Gram-negative bacteria and fungi.

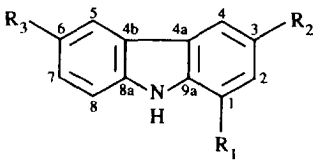
INTRODUCTION

Previously, some carbazole alkaloids were reported from *Clausena anisata* [1, 2]. The present investigation reveals the presence of two new carbazole alkaloids, clausenol and clausenine, from the alcoholic extract of the stem bark of *C. anisata*.

RESULTS AND DISCUSSIONS

Clausenol **1**, $C_{14}H_{13}NO_2$ ($[M]^+$ *m/z* 227) was found to be homogeneous by TLC and mass spectrometry. Zinc dust distillation of **1** led to the isolation of carbazole (**3**) and 3-methylcarbazole (**4**) indicating the presence of a 3-methylcarbazole residue in the molecule. A blue colouration with $FeCl_3$ indicated the presence of a phenolic hydroxyl group. Its UV spectrum λ_{max}^{EtOH} 228 (log ε 4.30), 253 (4.36), 303 (4.02), 356 nm (3.50) and IR spectrum ν_{max}^{KBr} 3395 (NH or OH) 1620, 1580 (aromatic system) 1204 ($ArOCH_3$) 975, 850, 820 cm^{-1} (substituted aromatic ring) suggested the presence of a carbazole nucleus. The ^1H NMR spectrum (100 MHz, DMSO-d_6) showed signals at δ10.56 (1H, *s*, OH proton, exchangeable with D_2O), 9.60 (1H, *bs*, NH proton, exchangeable with D_2O), 7.50 (1H, *d*, J = 2 Hz, H-4), 7.40 (1H, *d*, J = 2 Hz, H-5), 7.31 (1H, *d*, J = 8 Hz, H-8), 6.88 (1H, *dd*, J = 8 Hz, 2 Hz, H-7), 6.56 (1H, *d*, J = 1.5 Hz, H-2), 3.91 (3H, *s*, OCH_3) and 2.16 (3H, *s*, Ar-CH_3). Since the H-4 and H-2 protons were not *ortho*- coupled, positions 3 and 4 were substituted. The H-5 proton also was not *ortho*- substituted and slightly shielded, suggesting the position of the methoxyl group at C-6. The ^1H NMR signal for the hydroxyl proton of clausenol was very similar to that of a 1-hydroxyl group [3]. In the ^{13}C NMR spectrum of **1** (25 MHz, DMSO-d_6), the upfield shifts of the C-2 and C-4 in comparison to carbazole is also suggestive of the presence of a hydroxyl group at position 1.

On acetylation, **1** furnished an acetate **5**, mp 135°. The IR spectrum of **2** showed a strong peak at 1752 cm^{-1} for the acetoxy function and absence of the hydroxyl group. Reduction of the tosyl derivative of **1** with Raney nickel gave a compound **6**, mp 181°, which was identified as glycozoline [4] by direct comparison with an authentic sample. Since the structure of glycozoline has already been established as 6-methoxy-3-methylcarbazole, **1**, must have a methoxyl group at C-6 and a methyl group at the C-3 position. From all of this evidence, the structure of **1** was established as 1-hydroxy-6-methoxy-3-methyl carbazole, which was further supported by its ^{13}C NMR data. Finally, the structure of **1** was confirmed by synthesis (scheme 1).



1 R_1 = OH ; R_2 = Me ; R_3 = OMe
2 R_1 = R_3 ; OMe ; R_2 = Me
3 R_1 = R_2 ; R_3 = H
4 R_1 = R_3 ; H ; R_2 ; = Me
5 R_1 = OCOMe ; R_2 = Me ; R_3 = OMe
6 R_1 = H ; R_2 = Me ; R_3 = OMe

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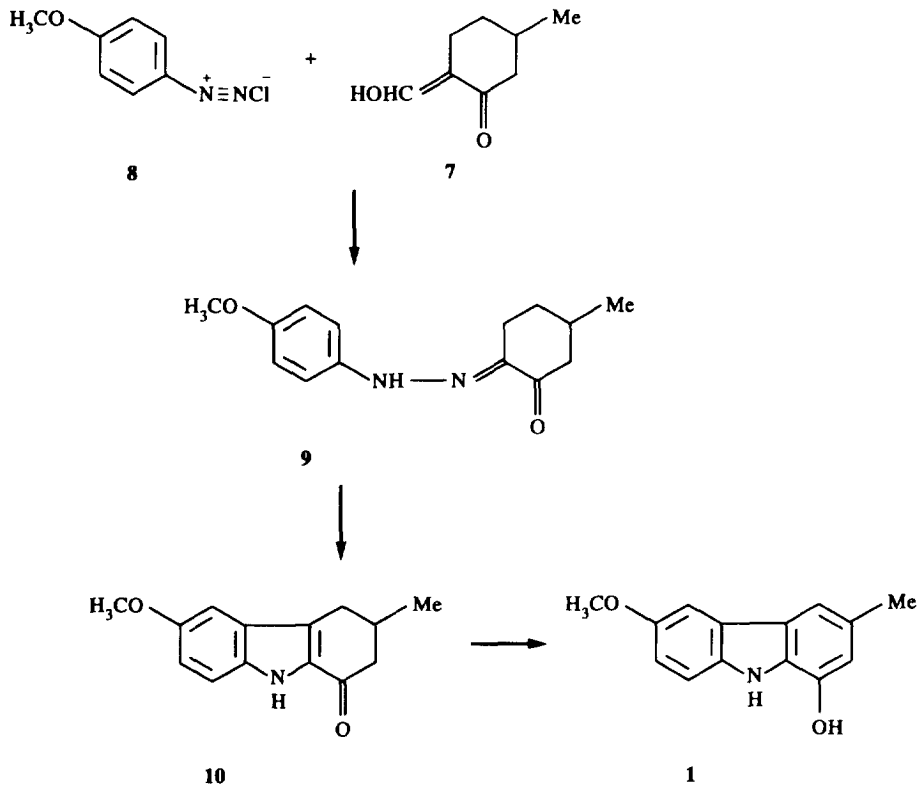

Scheme 1. Synthesis of clausenol (1).

2-Hydroxymethylene-5-methylcyclohexanone (7) [5] on condensation with 4-methoxybenzene diazonium chloride (8) under Japp–Klingemann conditions furnished 4-methylcyclohexane-1,2-dione-1-(4-methoxy) phenyl hydrazone (9, 49.5%), which on indolization with concentrated HCl and acetic acid furnished 6-methoxy-3-methyl-1-oxo-1,2,3,4-tetrahydrocarbazole (10, 64.5%). Compound 10 was refluxed with 10% Pd/C in declain and from the reaction product, the alkali-soluble fraction was separated and chromatographed over silica gel. Elution with CH_2Cl_2 furnished material (19.9%) identical with natural clausenol in all respects (mp, mmp, UV, IR, ^{1}H NMR).

Clausenine (2), $C_{15}H_{15}NO_2$ ([M]$^+$ m/z 241) had a UV spectrum λ_{max}^{EtOH} 226 (log ε 4.34), 242 (4.32), 299 (4.00), 340 (3.44), 354 nm (3.42), and IR spectrum ν_{max}^{KBr}3400 (– NH), 1580 (aromatic), 1218 (aromatic ether), 940, 840, 820 cm^{-1} (aromatic substitution) characteristic of a carbazole nucleus. The ^{1}H NMR (100 MHz, DMSO-d_6) showed signals at δ10.40 (1H, *s*, NH proton exchangeable with D_2O), 7.54 (1H, *d*, J = 2 Hz, H-4), 7.44 (1H, *d*, J = 2 Hz, H-5), 7.32 (1H, *d*, J = 8 Hz, H-8), 6.92 (1H, *dd*, J = 8 Hz, 2.5 Hz, H-7), 6.74 (1H, *d*, J = 1.5 Hz, H-2), 3.92 (3H, *s*, Ar-OCH_3), 3.80 (3H, *s*, Ar-OCH_3) and 2.42 (3H, *s*, Ar-CH_3). This spectrum was very similar to that of clausenol (1) except that instead of a hydroxyl proton, an additional methoxy signal (δ3.80) was apparent. It was therefore suggested that clausenine may be the methyl ether of clausenol. Methylation of 1 with diazomethane furnished a compound identical in all respects (mp, mmp, UV, IR, ^{1}H NMR) with clausenine. From this, the structure of 2, was established as 1,6-dimethoxy-3-methyl carbasole, which was further supported by its ^{13}C NMR spectrum (25 MHz, DMSO-d_6).

Studies on the antimicrobial properties of clausenol revealed that it was highly active against both Gram-positive and Gram-negative bacteria and fungi, while clausenine showed low activity against Gram-negative bacteria and fungi. The minimum inhibitory concentration (MIC) of clausenol and clausenine was determined by the agar dilution method. The MIC were studied up to 100 μg ml^{-1} and incubations were done at 37°. The MIC value of standard compounds, streptomycin (100 μg ml^{-1}), penicillin (2 μg ml^{-1}), nystatin and griseofulvin (1 μg ml^{-1}) were also studied. The MIC values are shown in Table 1.

EXPERIMENTAL

All mps are uncorr. UV and IR spectra were recorded in EtOH and as KBr pellets, respectively. ^{1}H and ^{13}C NMR were recorded at 100 and 25 MHz, respectively.

Isolation of alkaloids. Air-dried powdered stem bark (1 kg) of *C. anisata* was extracted with petrol in a Soxhlet for 36 hr. After extraction, the stem bark was dried and

Table 1. Minimum inhibitory concentrations of clausenol and clausenine

Sl. No.	Name of the organism	Compounds (MIC μg ml)					
		Clausenol	Clausenine	P	S	G	N
1	*Escherichia coli* ST 203	7	40	—	5	—	—
2	*Becillus subtilis* ST 204	14	> 100	0.01	—	—	—
3	*Salmonella typhi* ST 288	12	> 100	—	10	—	—
4	*Pseudomonas aeruginosa* ST 243	14	16	—	6	—	—
5	*Staphylococcus aureus* MC 27927	1.3	> 100	0.03	—	—	—
6	*Candida albicans* ST 388	5	16	—	—	—	0.05
7	*Trychophyton ruburm* ST 389	2	5	—	—	0.02	—

Key: — = Not tested; P = penicillin; S = streptomycin, G = griseofulvin, N = nystatin.

re-extracted with EtOH for 30 hr. The extract was freed from the solvent and the residue taken up in $CHCl_3$ and septd into acidic, basic and neutral frs. The acidic fr. was concd and chromatographed over silica gel (500 g). The column was eluted with petrol, petrol–CH_2Cl_2 (1:1), $CHCl_3$ and finally with a 2% $CHCl_3$–MeOH mixt. From the $CHCl_3$ eluate, clausenol (**1**, 120 mg, 0.012%) was obtained, which was recrystallized from benzene, mp 139°. (Found: C, 74.23; H, 5.65; N, 6.34%. Calcd for $C_{14}H_{13}NO_2$: C, 73.99; H, 5.77; N, 6.16%). ^{13}C NMR: δ 145.5 (C-1, *s*), 108.0 (C-2, *s*), 124.1 (C-3, *s*), 121.5 (C-4, *d*), 115.5 (C-4a, *s*), 116.0 (C-4b, *s*), 102.9 (C-5, *d*), 154.3 (C-6, *s*), 115.4 (C-7, *d*), 112.3 (C-8, *d*), 135.0 (C-8a, *s*), 134.6 (C-9a, *s*), 21.0 (Me, *q*). On concn of the neutral fr. it was subjected to CC over silica gel (400 g). The column was eluted with the same solvents as described above. From the petrol–CH_2Cl_2 (1:1) eluate a light yellow semi-solid mass was obtained. This was subjected to prep. TLC on silica gel G (1 mm thick) eluting with benzene-$CHCl_3$ (9:1). A major band (Rf 0.32) was sepd and extracted with $CHCl_3$. The residue obtained after removal of solvent was recrystallized from benzene–petrol, when crystals of **2** (170 mg, 0.042%), mp 151° were obtained. (Found: C, 74.99; H, 6.17; N, 5.95%. Calcd. for $C_{15}H_{15}NO_2$: C, 74.67; H, 6.27; N, 5.80%). ^{13}C NMR: δ 145.5 (C-1, *s*), 107.5 (C-2, *d*), 126.5 (C-3, *s*), 127.5 (C-4, *s*), 122.5 (C-4a, *s*), 123.6 (C-4b, *s*), 102.8 (C-5, *d*), 153.5 (C-6, *s*), 115.4 (C-7, *d*), 112.4 (C-8, *d*), 134.5 (C-8a, *s*), 135.1 (C-9a, *s*), 55.5 (1-OMe, *q*), 55.5 (6-OMe *q*).

Zinc dust distillation of **1**. Compound **1** (100 mg) was thoroughly mixed with Zn dust (10 g) previously dried at 250° and heated in a sealed tube for 2 hr. The ether-sol. portion of the reaction product was dissolved in benzene and chromatographed on alumina (5 g). Elution with benzene–petrol furnished a solid (5 mg) which showed the presence of two compounds by TLC on silica gel (petrol–HOAc, 10:1, Rfs 0.55 and 0.65, respectively). The two compounds were identified as, carbazole (mp 242°) and 3-methylcarbazole (207°), respectively by GC and HPLC [6, 7].

Acetylation of **1**. Compound **1** (70 mg) was dissolved in dry pyridine (2 ml) and Ac_2O (2 ml) and refluxed at 100° for 1 hr. After reaction the mixt. was poured into ice–H_2O (25 g) when a solid was obtained. The solid was filtered, washed with dil. HCl and finally with H_2O. It was then dried and recrystallized from benzene–petrol to give needles (55 mg, 66.3%) of **5**, mp 132°. UV: λ_{max}^{EtOH} 230.8 (log ε 4.40), 252.2 (4.13), 263.4 (3.98), 303.2 (4.18), 269 nm (3.45). IR: ν_{max}^{KBr} 3400 (-NH), 1752 cm^{-1} (– $OCOCH_3$). (Found: C, 71.75; H, 5.70; N, 5.39%. Calcd for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20%).

Tosylation and reduction of tosyl derivative of **1**. The tosyl derivative (50 mg) of **1**, prepd by the usual method, was dissolved in EtOH (6.5 ml) and refluxed in the presence of Raney Ni (0.2 g) for 2 hr. The solid obtained after work-up was purified by sublimation under vacuum followed by recrystallization from benzene–petrol when crystals of **6** (15 mg, 54.1%), mp 180°, were obtained. The compound was found to be identical with glycozoline in all respects (mp, mmp, UV, IR).

4-Methylcyclohexene – 1,2-dione-1-(4-methoxy)phenylhydrazone (**9**). 2-Hydroxymethylene-5-methylcyclohexanone (5.6 g) in MeOH (50 ml) was added to aq. NaOAc (8.10 g in 30 ml H_2O). To this soln was added an aq. acid soln of 4-methoxyphenyldiazonium chloride (prepd from 4.85 g of 4-methoxyaniline) during 30 min with mechanical agitation to afford a red ppt. of **9**. This ppt was filtered and washed with H_2O to remove acid. The product was recrystallized from EtOH when red needles of **9** (4.8 g, 49.8%), mp 191°, were obtained. (Found C, 68.66;

H, 7.57; N, 11.20%. Calcd. for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37). IR: ν_{max}^{KBr} 3400 (− NH), 1650 (> C = O), 1610 cm^{-1}.

6-Methoxy-3-methyl-1-Oxo-1,2,3,4-tetrahydrocarbazole (**10**). Compound **9** (3 g) was added to boiling HOAc (25 ml) and conc. HCl (6 ml) added through the reflux condenser. The soln was boiled for 3 min and then dild with ice–H_2O (150 ml). The solid obtained was filtered, washed with H_2O, dried and recrystallized from benzene to afford light ash-coloured crystals of **10** (1.8 g, 64.5%), mp 192°. (Found: C, 73.44; H, 6.61; N, 6.08, Calcd. for $C_{14}H_{15}NO_2$ C, 73.34, H, 6.59, N, 6.11%). UV: λ_{max}^{EtOH} 212.4 (log ε 4.21), 313 nm (435. IR: ν_{max}^{KBr} 3300 (− NH), 1635 (> C = O).

1-Hydroxy-6-methoxy-3-methylcarbazole (**1**). Compound **10** (1.5 g) was heated under reflux with Pd/C (10%, 0.5 g) in decalin (20 ml) for 5 hr. After reaction, decalin was removed by dist. under vacuum. The residue was dissolved in $CHCl_3$–MeOH (49:1) and filtered. The filtrate on evapn gave a brown residue (0.5 g), which was dissolved in Et_2O and extracted (× 3) with aq. NaOH (2%, *w/v*). The aq. alkaline soln on acidification with HCl gave a brown solid, which was subjected to CC over silica gel (10 g). The column was eluted with petrol, CH_2Cl_2 and $CHCl_3$, and the CH_2Cl_2 frs yielded a light brown solid which on recrystallization from benzene–petrol furnished light brown crystals, mp 138°, of **1** (0.35 g, 19.9%).

Methylation of **1** *and formation of* **2**. A MeOH soln (50 ml) of **1** (80 mg) was kept at 4°C for 24 hr with CH_2N_2. On removal of solvent, a residue was obtained which was washed with 2% aq. NaOH. The solid obtained was recrystallized from benzene–petrol to yield needles of **2** (65 mg, 90.6%), mp 150°.

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