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CARBAZOLE ALKALOID WITH ANTIMICROBIAL ACTIVITY FROM *CLAUSENA HEPTAPHYLLA*

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Key Word Index—*Clausena heptaphylla*; Rutaceae; clausenal; carbazole alkaloid; antimicrobial.

Abstract— A new carbazole alkaloid designated as clausenal was isolated from the leaves of *Clausena heptaphylla* and its structure established as 1,8-dimethoxy-3-formylcarbazole from physical, chemical and synthetic evidence. The alkaloid was found to be active against both Gram-positive and Gram-negative bacteria, and fungi.

INTRODUCTION

In the course of our investigations on carbazole alkaloids of *Clausena heptaphylla* [1], a new carbazole alkaloid designated as clausenal has been isolated from the neutral fraction of the ethanol extract of the leaves of the plant. Clausenal showed promising activity against both bacteria and fungi.

RESULTS AND DISCUSSION

Clausenal (**1**), $C_{15}H_{13}NO_3$ ($[M]^+$ *m/z* 255), mp 198°, was homogenous by TLC and mass spectrometry. It gave a 2,4-dinitrophenylhydrazone test and reduced ammoniacal $AgNO_3$ solution showing the presence of an aldehyde function. Its UV spectrum λ_{max}^{EtOH} 231 (log ε 4.45), 257 (4.09), 261 (4.10) and 327 nm (3.7)was characteristic of a 3-formyl carbazole [2]. The IR spectrum showed bands at ν_{max}^{KBr} 3400 (NH), 1660 (aldehyde), 1610, 1576 (aromatic system) and 1208 cm^{-1} (aromatic ether). In the ^1H NMR (100 MHz, $CDCl_3$) a signal at δ10.28 (*s*), and in the ^{13}C NMR (25 MHz; $CDCl_3$) a signal at δ191.4 (*d*) showed the presence of a formyl group. Besides, the ^1H NMR also showed signals at δ8.80 (1H, *br s*, NH, exchangeable with D_2O) 8.60 (1H, *d*, *J* = 2.8 Hz, H-4), 7.78 (1H, *dd*, *J* = 8 Hz and 1.8 Hz, H-5) 7.44 (1H, *d*, *J* = 1.8 Hz, H-2), 7.25 (1H, *m*, H-6), 6.98 (*dd*, *J* = 8 Hz and 1.8 Hz, H-7), 4.02 (3H, *s*, ArOMe) and 4.07 (3H, *s*, ArOMe).

Signals for H-4 and H-5 of carbazole alkaloids appeared at lower field as they were mutually deshielded. [3]. The relatively deshielded signal at δ8.60 was assigned to H-4, *ortho* to the aldehyde group. The appearance of this signal as a *meta*-coupled doublet suggested substitution at C-1 and C-3. The signal at δ7.44 attributed to H-2 also appeared as a *meta*-coupled doublet suggesting substitution at C-1 and C-3. One of the methoxy groups must, therefore, occupy position C-1. The signal for H-5 was *ortho*- and *meta*-coupled, showing that positions C-6 and C-7 are unsubstituted. Again the signal for H-6 appeared as a multiplet and that of H-7 as an *ortho*- and *meta*-coupled doublet. The second methoxy group, therefore, occupied position C-8. From these data the structure of clausenal was assigned as 1,8-dimethoxy-3-formyl carbazole which was also supported by its ^{13}C NMR spectrum (see Experimental).

The mass spectrum of **1** exhibited a base peak at *m/z* 255 ($[M]^+$). An intense peak at *m/z* 240 $[M - 15]^+$, together with a peak at *m/z* 212 $[M - 43]^+$, indicated the presence of a methoxy group [4]. A fragment peak at *m/z* 227 $[M - 28]^+$ supported the formyl carbazole skeleton in **1**.

Finally, the assigned structure was confirmed by synthesis as follows. 2-Hydroxymethylene-5-methylcyclohexanone (**2**) [4] on condensation with diazotized 2-methoxy aniline (**3**) under Japp–Klingemann conditions furnished 4-methylcyclohexane 1,2-dione 1-(2-methoxy)-phenyl hydrazone (**4**) which on cyclization with a mixture of acetic acid and conc. HCl furnished 8-methoxy-3-methyl-1-oxo-1,2,3,4 tetrahydrocarbazole (**5**). Dehydrogenation of **5** with 10% pd–C in boiling decatin furnished 1-hydroxy-8-methoxy-3-methyl carbazole (**6**), which on methylation with diazomethane furnished 1,8-dimethoxy 3-methylcarbazole (**7**). DDQ oxidation of **7** furnished **1** which was found to be identical with natural clausenal in all respects (mp, mmp, UV, IR and ^1H NMR).

Studies on the antimicrobial properties of clausenal revealed that it was active against both Gram-positive and Gram-negative bacteria, and fungi. The minimum inhibitory concentration (MIC) of clausenal was determined by the agar dilution method. The MIC was studied up to 100 μg ml^{-1} of the compound and incubation was done at 37°. MIC values for clausenal are shown in Table 1.

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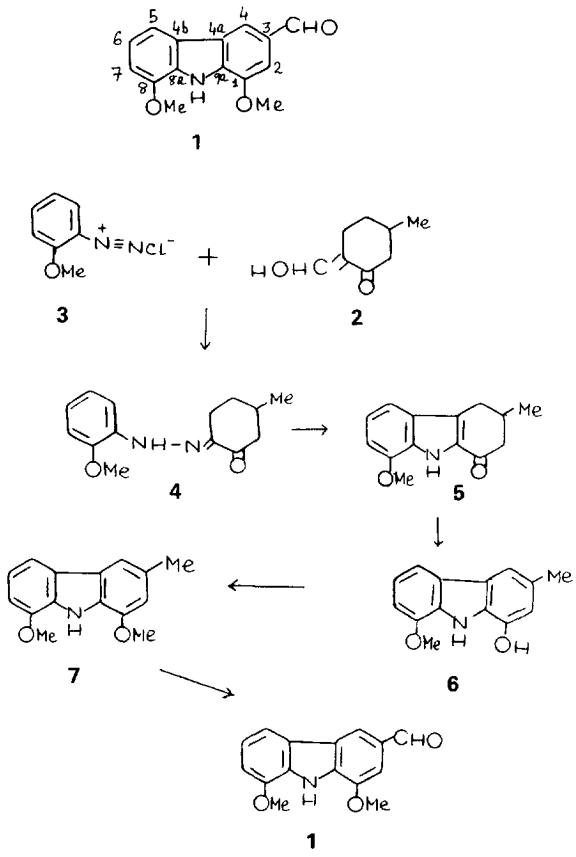

Table 1. Minimum inhibitory concentration for clausenal (1)

Organism	MIC ($\mu g\ ml^{-1}$)
1. *Escherichia coli* ST 203	6
2. *Bacillus subtilis* ST 204	18
3. *Salmonella typhi* ST 288	25
4. *Pseudomonas aeruginosa* ST 243	20
5. *Staphylococcus aureus* MC 27927	3
6. *Candida albicans* ST 388	8
7. *Trychophyton ruburm* ST 389	3

EXPERIMENTAL

Mps.: uncorr. UV and IR spectra were recorded in EtOH and as KBr pellets, respectively.

Isolation of clausenal. Air-dried, finely powdered leaves of *C. heptaphylla* (1 kg) were extracted with petrol in a Soxhlet for 36 hr. The solvent was then distilled off and the leaf dried and reextracted with 95% EtOH for 36 hr. From the EtOH extract, the solvent was evapd in a rotary evaporator. The residue was dried to yield a brown mass which was transferred to a thimble and extracted in a Soxhlet with CH_2Cl_2 for 30 hr. The solvent was distilled off from the CH_2Cl_2 extract to yield a brown residue (5 g). This was taken up in Et_2O and fractionated into neutral, acidic and basic frs in the usual way. The neutral fr. was then chromatographed over silica gel (200 g), eluting with petrol (60–80°), petrol–CH_2Cl_2 (1:1), CH_2Cl_2 and $CHCl_3$. The CH_2Cl_2 frs on removal of solvent furnished a light yellow solid (200 mg) which was rechromatographed over silica gel (10 g). The CH_2Cl_2 eluates gave on removal of solvent, a light yellow solid which was further purified by prep. TLC (silica gel G, 1 mm, benzene–$CHCl_3$, 9:1). The band of R_f 0.36 was sepd and extracted with 5% MeOH in $CHCl_3$. The residue on removal of solvent gave a solid (55 mg), which on recrystallization from benzene–petrol, furnished clausenal (**1**) as needles (40 mg), mp 198°. (Found C 70.90, H 4.97, N 5.60%; calculated for $C_{15}H_{13}NO_3$, C 70.58, H 5.13, N 5.49%). ^{13}C NMR (25 MHz, $CDCl_3$): δ146.1 (C-1, *s*), 102.8 (C-2, *d*), 128.4 (C-3, *s*), 120.6 (C-4, *d*), 123.6 (C-4a, *s*), 124.0 (C-4b, *s*), 112.2 (C-5, *d*), 119.8 (C-6, *d*), 104.9 (C-7, *d*), 145.6 (C-8, *s*), 120.1 (C-8a, *s*), 134.4 (C-9a, *s*), 55.5 (OMe-2, *q*), 194.4 (CHO, *d*).

4-Methylcyclohexane-1,2-dione-1-(2-methoxy)-phenylhydrazone (**1**). 2-Hydroxymethylene-5-methyl-cyclohexanone (5 g) in MeOH (60 ml) was added to an aq. soln of NaOAc (5.5 g in 35 ml H_2O). To this soln was added a soln of 2-methoxy phenyl diazonium chloride (prepd from 3 g of *o*-anisidine) during 30 min under mechanical agitation, when red crystals of **4** were obtained. The crystals were filtered off, washed with H_2O, dried and recrystallized from MeOH to give red needles of **4**, mp 91°. Yield 80% (Found C 68.66, H 7.57, N 11.20%, Calculated for $C_{14}H_{18}N_2O_2$, C 68.27, H 7.37, N 11.37%). IR ν_{max}^{KBr} cm^{-1} 3450 (NH), 1630 (CO) and 1600, 1510 (aromatic) cm^{-1}.

8-Methoxy-3-methyl-1-oxo-1,2,3,4-tetrahydrocarbazole (**5**). Compound **4** (2 g) was added to boiling HOAc (15 ml) and conc. HCl (3 ml) was added through the reflux condenser. The soln was boiled for 3 min and then poured into ice-H_2O (100 ml). The solid obtained was filtered, washed with H_2O, dried and recrystallized from benzene to yield light yellow crystals of **5**, mp 170°. Yield 67% (Found C 73.41, H 6.58, N 6.08%, Calculated for $C_{14}H_{15}NO_2$, C 73.36, H 6.55, H 6.11%. UV λ_{max}^{EtOH} nm 206, 245 and 305 nm, with log ε 3.96, 4.06 and 3.99. IR ν_{max}^{KBr} cm^{-1}: 3350 (NH) and 1630 (CO) cm^{-1}.

1-Hydroxy-8-methoxy-3-methylcarbazole (**6**). Compound **5** (0.8 g) was heated under reflux with 10% Pd/C (0.4 g) in decalin for 5 hr. After reaction, the soln was cooled and filtered. The residue, which contained crystals of the reaction product together with Pd/C, was dissolved in 2% MeOH in $CHCl_3$. The resulting mixt. was filtered to remove Pd/C. The filtrate, on evapn of solvent, gave a solid residue which was recrystallized from benzene to furnish crystals of **6**, mp 181°. Yield 68% (Found C 74.26, H 5.97, N 6.30%, Calculated for $C_{14}H_{13}NO_2$, C 73.99, H 5.77, N 6.16%). UV λ_{max}^{EtOH} nm: 242, 275, 284, 321 and 334 nm with log ε, 4.35, 3.39, 3.37, 3.36 and 3.38. IR ν_{max}^{KBr} cm^{-1} 3400 (NH), 3300 (OH) and 1590 (aromatic).

1,8-Dimethoxy-3-methylcarbazole (**7**). Ethereal CH_2N_2 at 0–5° (prepd from 4.5 g nitromethyl urea) was added to a soln of **6** (0.4 g) in MeOH (50 ml) kept at 0–5°. The mixt. was kept at 5° for 48 hr. After decomposition of excess CH_2N_2 with HOAc and removal of the solvent, a yellow semi-solid mass was obtained. This was dissolved in Et_2O

and washed with 1% NaOH soln followed by H_2O and then dried (Na_2SO_4). Removal of Et_2O gave a yellow solid which was recrystallized from benzene–petrol to yield needles of **7** (0.32 g), mp 161°. Yield 85% (Found C 74.99, H 6.17, N 5.60%, Calculated for $C_{15}H_{15}NO_2$. C 74.67, H 6.27, N, 5.80%), UV λ_{max}^{EtOH} nm: 242, 274, 283, 319 and 332 nm, log ε 4.33, 3.40, 3.23, 3.31 and 3.34. IR ν_{max}^{KBr} cm^{-1}: 3410 (NH) and 1590 (aromatic) cm^{-1}.

1, 8-*Dimethoxy*-3-*formylcarbazole* (*clausenal* **1**). A soln of **7** (200 mg) in fr. distilled dry benzene (60 ml) was stirred with DDQ (210 mg) at room temp. for 1 hr. The reaction mixt. was then washed with 10% HCl followed by H_2O, dried (Na_2SO_4) and the solvent removed. The residue was chromatographed on silica gel G. CH_2Cl_2 eluates on evapn of solvent furnished a light yellow solid which on recrystallization from benzene–petrol afforded **1**, mp 198°. Yield 35% (Found C 70.56, H 4.85, N 5.42%, Calculated for $C_{15}H_{13}NO_3$, C 70.58, H 5.13, N 5.49%).

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