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STRUCTURES OF CLAUSENIN AND CLAUSENIDIN TWO NEW PYRANOCOUMARINS FROM THE ROOTS OF *CLAUSENA HEPTAPHYLLA* Wt. & Arn.*

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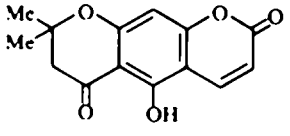
Abstract— From the roots of *Clausena heptaphylla*, Wt. & Arn., two pyranocoumarins have been isolated. On the basis of spectral and degradative evidence they have been constituted as I and IV respectively.[1]

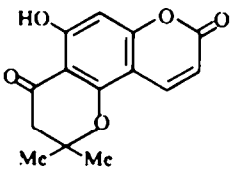
Clausena heptaphylla (Rutaceae) grows in the Western Ghats of India. The roots gave on hexane extraction, clausenin $C_{14}H_{12}O_5$, m.p. 156–157° and clausenidin $C_{19}H_{20}O_5$, m.p. 135–136°.

Both the compounds contain a chelated phenolic OH group as shown by the blue ferric colour and a bathochromic shift in the UV on addition of $AlCl_3$ to the ethanolic solution. IR spectra of clausenin and clausenidin show peaks at 1728, 1624, 1600 (coumarin) and 1639 cm^{-1} (chelated carbonyl). They form monomethyl ethers, and *p*-toluenesulphonates whose UV spectra resemble closely, xanthoxyletin. Alkaline hydrolysis provides the corresponding coumaric acids from which it is clear that both the compounds contain the coumarin nucleus.

Clausenin on KOH fusion gave phloroglucinol. On the basis of the above evidence and the informative NMR spectrum (Fig. 1-A), clausenin can be constituted as I or II. Unambiguous synthesis of I and II proved that clausenin possesses the linear pyranocoumarin structure I.[2]



I



II

The NMR spectrum of clausenidin (Fig. 1-B) shows that it contains an α,α-dimethyl-allyl group.

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[1] For a preliminary communication, see B. S. Joshi and V. N. Kamat, *Tetrahedron Letters* 5767 (1966).
[2] A. K. Ganguly, B. S. Joshi, V. N. Kamat and A. H. Manmade, *Tetrahedron* 23, 4775 (1967).

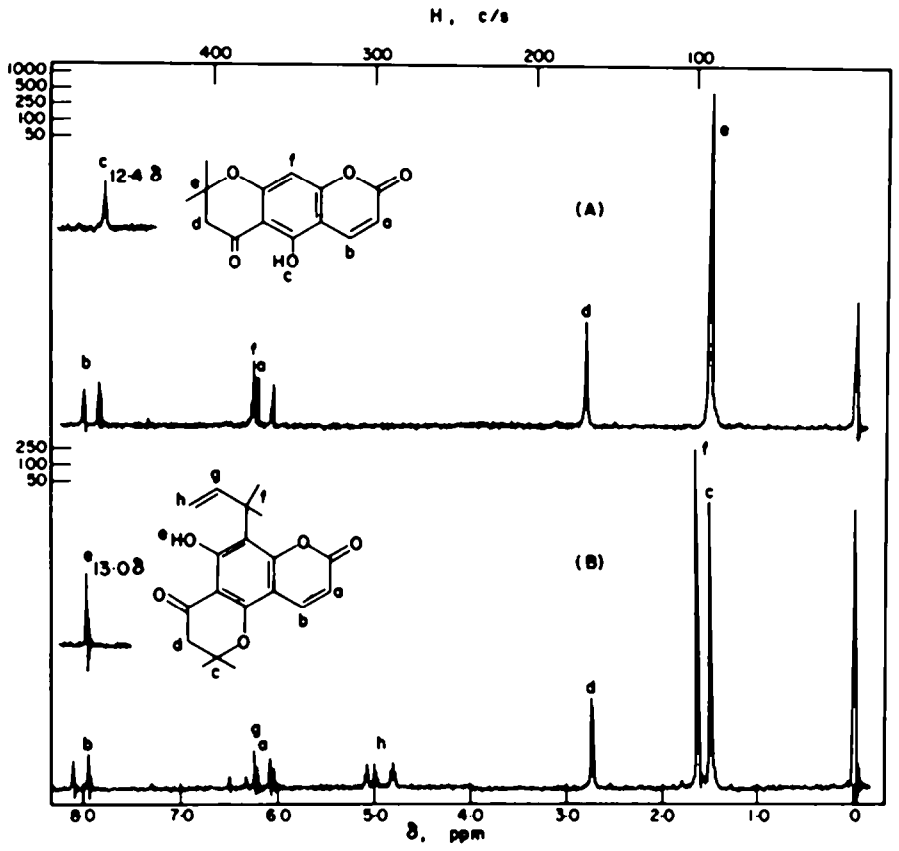
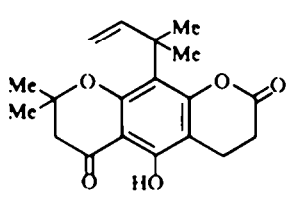
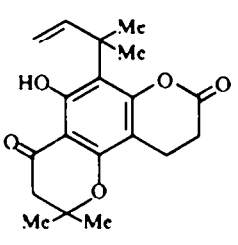


FIG. 1

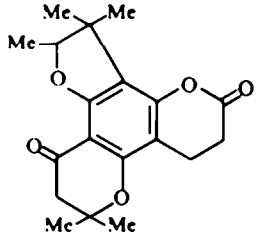
This evidence narrows the possible structures for clausenidin to III and IV.



III



IV



V

Clausenidin on treatment with sulphuric acid gave cycloclausenidin (V) and on heating with $AlCl_3$ in benzene it gave a hydroxypyranocoumarin, m.p. 220° which was found to be identical with the synthetic compound II.[2] Clausenidin should therefore be constituted as IV.

Clausenin and clausenidin are the only examples of naturally occurring coumarins fused to a dimethylpyranone ring. The relationship of clausenin with xanthoxyletin

is of biogenetic interest. Clausenidin is the first example of a natural coumarin bearing an α,α-dimethyl allyl group.

EXPERIMENTAL

UV spectra were taken on a Beckman DU Spectrophotometer. IR spectra were taken on a Perkin-Elmer Model 421 Spectrophotometer. NMR spectra were taken using a Varian A-60 spectrometer in $CDCl_3$ solutions with TMS internal reference standard. M.ps are uncorrected.

Isolation of clausenin I. The powdered roots (12 kg) were soaked with hexane (30 l.) for 12 hr and the material re-extracted with fresh hexane (25 l.). The two extracts were combined, and concentrated to 300 ml. The concentrate on standing overnight deposited clausenin. Recrystallization from CH_2Cl_2–pentane gave colourless needles (2 g) m.p. 157 158°. TLC on Sigel (R_f 0·37, benzene–Chf 1:1). λ_{max}^{alc} 216, 279, 320 mμ (log ε 4·14, 4·44, 4·08). IR (Nujol) 1728, 1639, 1624, 1600, 1569, 1310, 1270, 1224, 1201, 1159, 1118, 1089, 920, 865, 820, 795, 780 and 697 cm^{-1}. (Found: C, 64·7; H, 4·7. Mol. wt. by mass spectrum 260. $C_{14}H_{12}O_3$ requires: C, 64·6; H, 4·7%. Mol. wt. 260.)

Isolation of clausenidin (IV). The roots after extraction of clausenin were further extracted thrice with boiling hexane (60–80°). The combined extracts along with the mother liquor from the isolation of clausenin were concentrated under reduced press. The oil (60 g) dissolved in benzene (100 ml) was chromatographed over a column of Sigel (600 g, 0·2–0·5 mm) with benzene as eluent. Fractions (100 ml) were collected, and the progress of the chromatogram followed by TLC. Fractions 18–47 which showed a single spot (R_f 0·61, benzene–Chf 1:1, yellow fluorescence in UV light), were combined and the solvent removed. The residue on crystallization from CH_2Cl_2–hexane gave clausenidin as yellow prismatic rods (1·5 g), m.p. 136–137°. λ_{max}^{alc} 222, 284 and 328 mμ (log ε 4·23, 4·53 and 4·11). IR (Nujol) 1728, 1639, 1600, 1310, 1292, 1230, 1180, 1170, 1150, 1125, 1102, 1090, 988, 970, 905, 855, 790 and 730 cm^{-1}. (Found: C, 69·5; H, 6·1 Mol. wt. by mass spectrum 328. $C_{19}H_{20}O_5$ requires: C, 69·5, H, 6·1%. Mol. wt. 328.)

Clauseninmonomethyl ether. To a soln of clausenin (2·1 g) in MeOH (60 ml) and ether (600 ml) was added an excess of diazomethane, and the reaction mixture allowed to stand at room temp for 6½ hr. Excess diazomethane was decomposed with a few drops of AcOH and the solvent removed. A gummy residue was obtained, which crystallized on trituration with ether. The crude product was collected and recrystallized from CH_2Cl_2–ether (600 mg), m.p. 149 150°. TLC on Si gel (R_f 0·13, benzene–Chf 1:1). λ_{max}^{alc} 220, 262, 315 and 340 mμ (log ε 4·16, 4·39, 4·11 and 4·04). IR (Nujol) 1729, 1691, 1611, 1599, 1560, 1420, 1330, 1310, 1280, 1252, 1215, 1190, 1148, 1120, 1100, 1088, 1065, 972, 950, 910, 888, 855, 835, 818, 788, 772, 712, 678 and 655 cm^{-1}. NMR 6·2, 7·95 (d, 1-H each, J = 10 c/s, C-3, C-4 protons of coumarin), 6·61 (S, 1 H, aromatic), 4·0 (S, —OCH_3), 2·75 (s, —CH_2—), 1·51 δ (s, $>C(Me)_2$). (Found: C, 65·5; H, 5·4. $C_{15}H_{14}O_4$ requires: C, 65·7, H, 5·2%.) Clauseninmonomethyl ether formed a crystalline 2,4-dinitrophenyl hydrazone, m.p. 265–267° (dec). (Found: C, 56·1; H, 4·5; N, 12·7; $C_{12}H_{18}N_4O_8$ requires: C, 55·5; H, 4·0; N, 12·3%.)

Clausenindimethylallyl ether. A mixture of clausenin (130 mg), dimethylallyl bromide (100 mg) anhyd K_2CO_3 (300 mg) and dry acetone (10 ml) were refluxed on a water bath for 24 hr. The solvent was removed under reduced press, and the residue extracted thrice with cold ether. The solvent was removed and the gummy residue crystallized from ether pentane (55 mg), m.p. 118–121°. TLC on Si gel (R_f 0·6, benzene–Chf 2:3). λ_{max}^{alc} 266, 315 mμ (log ε 4·35, 4·09). IR (Nujol) 1745, 1692, 1608, 1591, 1565, 1335, 1325, 1308, 1280, 1240, 1215, 1195, 1145, 1111, 1085, 1030, 978, 935, 905, 842, 825, 780, 760, 715 and 680 cm^{-1}. NMR 6·25, 8·0 (d, 1-H each, J = 10 c/s), 6·62 (s, 1-H aromatic), 5·6 (broad triplet, 1-H), 4·67 (d, —OCH_2—), 2·78 (s, —CH_2—), 1·8 (S, —CH_3), 1·72 (S, —CH_3) and 1·5 δ (S, 6H, $>C(Me)_2$). (Found: C, 69·4; H, 6·1. $C_{19}H_{20}O_5$ requires: C, 69·5; H, 6·1%.)

Clausenin-p-toluenesulphonate. To a soln of clausenin (260 mg) in dry pyridine (5 ml) was added *p*-toluenesulphonyl chloride (260 mg) and the reaction mixture kept at room temp for 24 hr. It was diluted with 2N HCl and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with 5% $NaHCO_3$ aq and water dried over Na_2SO_4. Evaporation of the solvent gave a colourless gum, which crystallized from CH_2Cl_2–EtOH as colourless prismatic rods (290 mg), m.p. 195°. TLC on Si gel (R_f 0·56, Chf). λ_{max}^{alc} 220, 258, 278,

310 and 334 mμ (log ε 4·45, 4·27, 4·30, 4·05 and 4·05). IR (Nujol) 1742, 1692, 1622, 1602, 1552, 1332, 1310, 1280, 1225, 1195, 1180, 1160, 1125, 1108, 1098, 1058, 915, 895, 870, 860, 840, 818, 808, 785, 755, 725, 700 and 670 cm^{-1}. NMR 6·2, 7·7 (d, 1-H each, J = 10 c/s), 6·8 (s, 1-H, aromatic), 7·4–7·9 (4-H, aromatic), 2·72 (S, 2H), 2·5 (s, 3H, aromatic $-CH_3$), 1·48 (s, 6-H, $>C(Me)_2$). (Found: C, 61·2; H, 4·1; $C_{21}H_{18}O_7S$ requires: C, 60·9; H, 4·4%.)

Alkaline hydrolysis of clausenin. A soln of clausenin (200 mg) in MeOH (4 ml) and 1% KOH aq (32 ml) was refluxed for 30 min. The reaction mixture was cooled and acidified with 2N HCl when a semi-crystalline ppt was formed. This was allowed to stand overnight at room temp, filtered and washed free from acid with cold water. Recrystallized from ether (100 mg), m.p. 214–215° (dec). λ_{max}^{alc} 292 mμ (log ε 3·1). IR (Nujol) 3400, 1678, 1618, 1575, 1372, 1322, 1272, 1242, 1185, 1165, 1095, 1068, 991, 825, 805, 778, 758 and 712 cm^{-1}. (Found: C, 60·6; H, 5·0. $C_{14}H_{14}O_6$ requires: C, 60·4; H, 5·1%.)

Alkali-fusion of clausenin. Clausenin (2 g) was mixed with KOH (5 g) in a nickel crucible and heated at 250° for 1 hr. The product was cooled and digested with water. The brown soln was acidified with HCl and extracted with ether. The ether extracts were pooled, washed with 5% $NaHCO_3$ aq and H_2O, dried over Na_2SO_4 and concentrated to a small volume. A red brown gummy residue mixed with crystals was obtained. This was carefully diluted with dry ether and the crystals were separated by filtration and washing with cold ether (20 mg), m.p. 218°. Its identity with phloroglucinol was confirmed by mixed m.p., TLC and superimposable IR spectrum.

Dihydroclausenin. Clausenin (260 mg) was dissolved in EtOH (30 ml) and hydrogenated at 26° using Raney Ni catalyst (0·75 g). The reaction was stopped after 7 hr. After filtration of the catalyst, the solvent was removed under reduced press. The semicrystalline residue was extracted with ether which on slow evaporation deposited heavy prisms (52 mg), m.p. 159–160°. TLC on Si gel (R_f 0·65, benzene–Chf 2:3). λ_{max}^{alc} 212, 286 and 342 mμ (log ε, 4·31, 4·17, 3·47). IR (KBr) 1772, 1640, 1590, 1480, 1462, 1442, 1428, 1390, 1370, 1330, 1310, 1300, 1288, 1245, 1220, 1190, 1170, 1150, 1130, 1088, 1070, 1022, 985, 960, 910, 880, 848, 820, 795, 730, 675, 660, 635, 615 and 608 cm^{-1}. NMR 12·4 (S, 1-H, OH), 6·0 (S, 1-H, aromatic), 2·7 (S, 2H), 1·48 (S, 6H, $>C(Me)_2$). (Found: C, 64·3; H, 5·3. $C_{14}H_{14}O_5$ requires: C, 64·1; H, 5·4%.)

Clausenidinmonomethyl ether. To a soln of clausenidin (320 mg) in MeOH (10 ml) and ether (20 ml) was added an excess of diazomethane, and the reaction mixture allowed to stand at room temp for 6½ hr. Excess diazomethane was decomposed with a few drops AcOH and the solvent removed. A gummy residue was obtained, which showed a tendency to crystallize after standing at room temp for over a fortnight. It was dissolved in benzene and chromatographed on a column of Si gel using benzene as eluent. Fractions (5 ml) were collected and the progress of the chromatogram was followed by TLC. Fractions 24–31 were combined and the solvent removed. A semi-crystalline residue (110 mg) was obtained, which crystallized from pentane (27 mg), m.p. 86–87°. TLC on Si gel (R_f 0·14, benzene–Chf 2:3). IR (Nujol) 1740, 1690, 1612, 1570, 1410, 1330, 1290, 1220, 1160, 1140, 1115, 1090, 1075, 970, 955, 905, 890, 822, 785 and 720 cm^{-1}. (Found: C, 70·1; H, 6·3. $C_{20}H_{22}O_5$ requires: C, 70·2; H, 6·5%.)

Clausenidin-p-toluenesulphonate. To a soln of clausenidin (328 mg) in dry pyridine (7 ml) was added *p*-toluenesulphonyl chloride (328 mg) and the reaction mixture kept at room temp for 24 hr. It was diluted with CH_2Cl_2, washed successively with 2N HCl, 5% $NaHCO_3$ aq and H_2O and dried over Na_2SO_4. Evaporation of the solvent gave a gum which crystallized from CH_2Cl_2–EtOH as pale yellow needles (260 mg), m.p. 198°. TLC on Si gel (R_f 0·22, benzene–Chf 2:3). λ_{max}^{alc} 218, 283, 310 and 334 mμ (log ε 4·38, 4·26, 3·96 and 3·90). IR (Nujol) 1745, 1700, 1640, 1620, 1588, 1545, 1530, 1490, 1468, 1408, 1378, 1345, 1330, 1305, 1295, 1265, 1218, 1190, 1180, 1160, 1142, 1120, 1108, 1095, 1082, 1055, 1040, 975, 960, 932, 902, 888, 825, 810, 792, 780, 755, 728, 715 and 700 cm^{-1}. (Found: C, 64·7; H, 5·6. $C_{26}H_{26}O_7S$ requires: C, 64·7; H, 5·4%.)

Cycloclausenidin (V). Clausenidin (100 mg) was treated with conc H_2SO_4 (1 ml) and the cherry red soln allowed to stand at room temp for 30 min. It was then poured into water and extracted with CH_2Cl_2. The CH_2Cl_2-extract was washed with water, dried over Na_2SO_4 and the solvent removed. A gum was obtained, which crystallized from ether–pentane (30 mg), m.p. 152–153°. λ_{max}^{alc} 222, 272 and 340 mμ (log ε 4·18, 4·43 and 4·16). IR (Nujol), 1690, 1608, 1588, 1322, 1300, 1230, 1182, 1160, 1110, 1070, 1030, 970, 880, 865, 835, 795, 778 and 720 cm^{-1}. (Found: C, 69·3; H, 6·3. $C_{19}H_{20}O_5$ requires: C, 69·5; H, 6·1%.)

Cycloclausenidin formed a crystalline 2,4-dinitrophenylhydrazone, m.p. 283° (dec). (Found: C, 59·3; H, 5·1; N, 11·2. $C_{25}H_{24}N_4O_8$ requires: C, 59·1; H, 4·8; N, 11·0%.)

Alkaline hydrolysis of clausenidin. A soln of clausenidin (200 mg) in MeOH (4 ml) and 1% KOH (32 ml) was refluxed for 30 min. The product was cooled and acidified with 2N HCl when a ppt was obtained. This was extracted with ether, washed with water, dried over Na_2SO_4 and concentrated to a small volume. Fine needles deposited on standing (120 mg), m.p. 275–277° λ_{max}^{alc} 290 mμ (log ε 4·64). IR (Nujol) 3410, 1690, 1620, 1590, 1395, 1330, 1310, 1298, 1272, 1195, 1168, 1130, 1120, 1099, 1080, 1060, 1035, 991, 952, 938, 920, 885, 870, 855, 830, 782, 725, 715 and 650 cm^{-1}. (Found: C, 64·8; H, 6·4; $C_{19}H_{22}O_6$ requires: C, 64·7; H, 6·6%.)

Dealkylation of clausenidin to II. A soln of clausenidin (200 mg) in dry benzene (10 ml) was added to a suspension of anhyd $AlCl_3$ (300 mg) in dry benzene (5 ml). The reaction mixture was refluxed for 2 hr, cooled and poured into dil HCl (ice). The benzene layer was separated, washed with 5% $NaHCO_3$ soln, water, dried over Na_2SO_4 and the solvent removed (150 mg). This was dissolved in benzene and chromatographed on a Si gel column. Elution with benzene–Chf (1:1) gave a homogeneous substance (25 mg), m.p. 218–220°. A mixed m.p. with II was undepressed; the identity was further confirmed by identical TLC behaviour and superimposable IR spectra (KBr).

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