mately 3:1 ratio. The two isomers were just separable on Si gel plates developed with $CHCl_3$–EtOAc (1:1), R_f's columbin 0·48 and isocolumbin 0·38, or with CH_2Cl_2-MeOH formamide (80:19 1), R_f's 0 86 and 0 79 respectively The two compounds were detected with phosphomolybdic acid or vanillin-orthophosphoric acid

This is the first report of the natural occurrence of isocolumbin in plants. Preliminary tests indicate that it is as strongly bitter as columbin. The ecological importance of serendipity berry containing both intensely sweet and strongly bitter principles is as yet unresolved Tests carried out to see if columbin was an attractant or deterrent to the feeding of termites gave no positive results

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REFFRENCES

1 van Wel, H and Loeve, K (1973) *Fed Eur Biochem Soc Letters* **21**, 38
2 Inglett, G F (1975) in *The Chemistry and Biochemistry of Plant Proteins* (ed Harborne J B and van Sumere, C F eds), pp 265–280 Academic Press, London
3 Barton, D H R and Eland D (1956) *J Chem Soc* 2085
4 Wessely F, Dinjaski, K, Isemann W and Singer G (1935) *Monatsh* **66**, 87
5 Gilbert J N T Mathieson D W and Patel, M B (1967) *Phytochemistry* **6**, 135
6 Hsu, H Y Ruo T I Iguchi, K and Kakisawa, H (1973) *Phytochemistry* **12**, 3000
7 Ito, K and Furukawa, H (1969) *Chem Commun* 653

Phytochemistry 1975, Vol 14, pp 2720–2721 Pergamon Press Printed in England

A NEW CARBAZOLE ALKALOID AND COUMARINS FROM ROOTS OF *CLAUSENA ANISATA*

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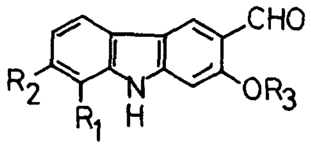
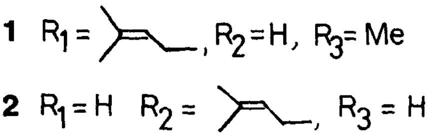
Key Word Index—*Clausena anisata*, Rutaceae, carbazole alkaloid clausanitin, coumarins, osthol, imperatorin, coumarrayin, chalepin

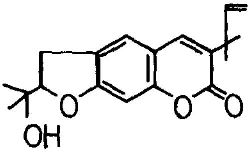
It has been reported[1] that the roots of *Clausena anisata* (Willd). Rutaceae contained the coumarins imperatorin and coumarrayin. Recently we isolated[2] a new carbazole alkaloid, atanisatin (**1**) from the stem. We now report a re-investigation of the roots which has led to the isolation of another new carbazole alkaloid, clausanitin (**2**) as well as 4 coumarins.

The gummy solid from hot hexane extraction of the roots was chromatographed on Si gel using increasing percentages of Et_2O in light petroleum (60–80°), to give three different crystalline fractions. The first (**2**) formed silky yellow crystals mp 154–156°; MS gave MW 279, $C_{18}H_{17}NO_2$ (Req. C, 77·4; H, 6·1; N, 5·0; found C, 77·2; H, 6·0; N, 5·0%). IR, ν_{max} 3400 (weak), 3300, 1635 (weak), 1610 cm^{-1} attributable to –OH, –NH, and H–bonded aromatic CO and aromatic ring respectively: $FeCl_3$ and ammoniacal $AgNO_3$ positive (phenol) and dinitrophenylhydrazine indicated the presence of CHO. It gave a positive carbazole test[3], and appeared to be a 3-formyl carbazole by UV: λ_{max} 239, 249 (sh), 278, 288 (sh), 297 and 340 nm (log ϵ = 4·40, 4·29, 4·39, 4·46, 4·53 and 3·90 respectively) in agreement with those of murrayanine[4] and atanisatin[2]. NMR (Varian A-60, $CdCl_3$, δ-scale, TMS as internal standard) showed two one-proton sharp singlets at 9·87 (–CHO); 11·75 (–OH), the latter disappearing on deuteration; 1·72, a three proton slightly split peak; 1·85 (*s*), three proton; 3·62 (*d*), two protons (*J* 7 Hz); 5·33 (*t*), one proton (*J* 7 Hz), representing 2 vinyl methyls, benzylic methylene and vinyl proton respectively probably indicating a prenyl group; other signals were three one-proton at 8·0(*s*) (C-4), 7·37(*s*) (C-8) and 7·34(*s*) (C-1), one proton 7·9(*d*) (*J* 7 Hz) (C-5) and a one proton 7·31(q) (*J* 7, 2 Hz) (C-6); accounting for 5 aromatic protons The –NH proton resonated at 7·85.

Since clausanitin (**2**) is a 3-formyl carbazole and the formyl group is chelated to the OH group, the latter must be at C-2 or C-4. Position C-2 is most likely on biogenetic grounds and if the OH group were at C-4, the NMR three one-proton sharp singlets at 8·0, 7·37 and 7·34 would not occur. The γ,γ-dimethylally group is obviously at C-7 on the basis of the NMR signals







3

for protons on C-4 and C-5[4, 5] and by the fact that the corresponding 6-derivative has been synthesised[5] and the reported spectral data and mp are different from those of (2).

The IR, UV and NMR of the second crystalline compound from the root were identical with those of imperatorin. The compound, which was the major fraction, gave no depression on mp with an authentic sample.

The third crystalline compound had mp 111–113°. Molecular weight, 314, (MS); and the elemental analysis gave $C_{19}H_{22}O_4$. The IR was different from those of imperatorin and coumarrayin, but had a strong CO absorption peak and a peak at 3400 cm^{-1} (OH); UV λ_{max} 295, 330 nm, log ϵ (3·64, 4·12). The NMR differed from those of imperatorin and coumarrayin in lacking doublets at 6·3 and 7·8; the vinylic one proton triplet (J 7 Hz) in the region δ 5·2–5·8 and any vinylic methyl groups characteristic of the presence of a γ,γ-dimethylallyl group and any furan protons. It showed 4 tertiary methyl groups at δ 1·23 (3H); 1·35 (3H) and 1·43 (6H); and a set of coupled peaks reminiscent[6] of the presence of an α,α-dimethyl allyl group. The hydroxy group which appeared at δ 2·16 (disappeared on exchange with D_2O) was shown to be tertiary since it could only be acetylated under forcing conditions to give an acetate mp 104–106°. More detailed NMR studies led to the structure (3) as that of chalepin mp 118–119° which was isolated[7, 8] from the roots of *Ruta chalepensis*. The reported[7] NMR sppeared to be identical with that of our compound.

Column chromatography of the hexane extract of another batch of roots of *Clausena anisata* from the same location gave four crystalline compounds The first, mp 84–86° was shown (UV, IR, NMR) to be osthol. The second, third and fourth crystalline compounds were found to be identical in all respects with coumarrayin, imperatorin and chalepin respectively.

It is significant to note that the 2 carbazole alkaloids and 3 out of the 4 coumarins so far isolated from *Clausena anisata* possessed the γ,γ-dimethylallyl group. The only exception, chalepin has the α,α-dimethyl allyl system.

EXPERIMENTAL

Extraction of the roots Powdered roots (3 3 kg) were extracted with refluxing hexane The concentrate gave a gummy solid (25 0 g) which was dissolved in C_6H_6 and chromatographed on a column of Si gel (100 g) elution with Et_2O–petrol (60–80°) Light petrol–Et_2O (8 2) eluted clausanitin (2) as silky yellow crystals mp 154–156° (0 032 g) Light petrol–Et_2O (7 3) fractions yielded, imperatorin mp 100–101° (2·34 g) Light petrol–Et_2O (6 4) eluates afforded brownish crystals of chalepin mp 111–113°(0 68 g) The second batch of roots treated similarly and light petrol–Et_2O (8·2) furnished osthol mp 84–86° and coumarrayin mp 153–155°; but no clausanitin, light petrol–Et_2O (7·3) gave imperatorin and light petrol–Et_2O (6 4) furnished chalepin

REFERENCES

1. Abe, M. O and Taylor, D A. H. (1971) *Phytochemistry* **10**, 1167.
2 Okorie, D A. (1975) *Tetrahedron Letters* (in press).
3 Joshi, B. S., Kamat, V N, Saksena, A. K., and Govindachari, T R (1967) *Tetrahedron Letters* 4019.
4. Chakraborti, D. P, Barman, B K and Bose, P K (1965) *Tetrahedron* **21**, 681.
5 Joshi, B. S, Kamat, V N, Gawad, D. H and Govindachari, T. R. (1972) *Phytochemistry* **11**, 2065
6 Reisch, J, Szendrei, K, Minker, E and Novak, I (1968) *Tetrahedron Letters* 4395
7 Brooker, R. M, Eble, J N. and Starkovsky, N. A (1967) *Lloydia* **30**, 73
8. Seshadri, T R and Vishwapaul, T R. (1973) *J Sci Ind. Res.* **32**, 227.