Phytochemistry, 1975, Vol 14, pp 2719 2720 Pergamon Press Printed in England

THE BITTER PRINCIPLES OF *DIOSCOREOPHYLLUM CUMMINSII* SEED

EQIL RAMSTAD,* JOHN W. POWELL† and BEVERLEY J. WILSON‡

*University of Ife, Ife, Nigeria, †University of London, and ‡Rhodes University, Grahamstown, South Africa

and

S. KOLA ADESINA, JOHN D. HIGGINBOTHAM and JEFFREY B. HARBORNE

Phytochemical Unit, Plant Science Laboratories, The University, Reading, England

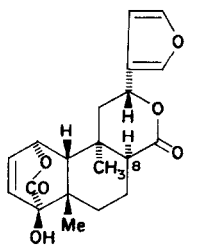
(*Revised Received* 5 *June* 1975)

Key Word Index—*Dioscoreophyllum cumminsii*, Menispermaceae, bitter principles, columbin, isocolumbin, diterpenoids

While the pericarp of the serendipity berry (*Dioscoreophyllum cumminsii* (Stapf) Diels, Menispermaceae) has a persistent, intensely sweet taste due to the content of a chemostimulatory, taste-active protein called monellin [1] or serendip [2], other parts of the fruit, particularly the seeds, taste bitter. This fact prevents the use of the whole fruit for sweetening purposes. Our two groups have now independently identified the bitter substance columbin in the seeds and those of us at Reading have also found isocolumbin to be present.

The fresh, fully ripe berries were collected near Ede, Nigeria, and the fleshy sarcocarp removed by rubbing and washing. The seeds, after drying, powdering and defatting with light petroleum, were extracted by cold percolation with EtOH. Upon evaporation of the extract, a copious white precipitate formed, which was recrystallized from $CHCl_3$ upon addition of light petrol. Recrystallization from acetone gave a yield of 0·3%, based on weight of fresh fruit, of colourless needles, mp 195–196° with effervescence, $[\alpha]_D^{20}$ +52·7° (*c* 1·0 in pyridine). (Found: C, 66·72; H, 6·47. $C_{20}H_{22}O_6$ requires: C,67·1; H, 6·20%.) This was identified as columbin (**1**) from study of the IR, NMR and MS data and from its ready loss of CO_2 on heating to furnish decarboxycolumbin, mp 140–141° [3]. This identification was confirmed by direct comparison (mmp, IR, NMR, MS) with an authentic specimen.

Originally isolated from colombo root (*Jateorhiza palmata* Miers, Menispermaceae) [4], columbin has more recently been found in seeds of *Sphenocentrum jollyanum* (Menispermaceae) [5]. This is thus the third report of its occurrence in this family. It has, however, been found once in an unrelated family, in *Melothria maderaspatana*



(**1**) Columbin
(**2**) Isocolumbin (8-C epimer)

Cogn. (Cucurbitaceae) [6], so that it cannot be regarded as being entirely specific to the Menispermaceae. Nevertheless, the occurrence of several oxides of columbin in another member of the family, *Fibraurea chloroleuca* [7], suggests that this type of diterpenoid bitter principle may be fairly widespread in this plant group.

The original crude bitter principle, before recrystallization, was found to contain a closely related second component, which could only be separated from columbin by repeated column chromatography on Si gel using $CHCl_3$–EtOAc (1:1). The slower moving second component was eventually obtained pure, following recrystallization from CH_2Cl_2–MeOH (4:1), as needles, mp 183–168°. (Found: C, 66·48; H, 6·16%.) It was identified as isocolumbin (**2**) by direct spectral and mp comparison (of the compound and its acetate) with authentic material (and acetate) prepared by isomerizing columbin with mild alkali [3].

Since the interconversion of columbin to isocolumbin (i.e. epimerization at C-8) occurs under mild conditions, it was possible that isocolumbin was simply an artifact of the isolation procedure. That this was not so was shown by TLC examination of a direct extract of fresh seeds, when columbin and isocolumbin were detected in approxi-

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

Acknowledgements– E R acknowledges a visiting fellowship from the CSIR of South Africa The work at Reading was financially supported in part by Messrs Tate and Lyle Ltd and the University of Ife, Nigeria We thank Mr Butters (Tate & Lyle) and Dr S Agurell (Astra Lakemedel AB, Soderstaffe, Sweden) for spectral measurements, Dr J N T Gilbert (Univ of London) for an authentic sample of columbin and Mr S L O Malaka (Univ of Ife Nigeria) for tests on termites

REFERENCES

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*

DOMINIC A. OKORIE
Department of Chemistry, University of Ibadan, Ibadan, Nigeria

(*Received* 10 *April*, 1975)

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