n. :ue can significantly improve the flow properties of aspirin without causing change in crystal form. The process would be a better alternative to slugging of moisture sensitive drugs. The agglomerates should be further subjected to evaluation of stability and compressibility.

The authors wish to thank University Grants Commission, New Delhi, for the sanction of minor research project to ARP, and to Li-Taka Pharmaceuticals, Pune for gift sample of aspirin.

REFERENCES

1. Paradkar, A.R., Pawar, A.P. Mahadik, K.R., and Kadam, S.S. **Indian drugs.**, 1994, 31, 229.

2. Kawashima Y., Cui, F., Takeuchi, H., Hino, T., Niwa. T., and Kiuchi, K., **Powder Technology.**, 1984, 78, 151.

3. Kawashima, Y., Niwa, T., Takeuchi, H., Hino, T. and Ito, Y., **Chem. Pharm. Bull.**, 1992, 40, 196.

4. Niwa, T., Takeuchi, H., Hino, T., Itoh, A., Kawashima, Y., and Kiuchi, K. **Pharm. Res.**, 1994, 11, 478.

5. Udea, M., Nakamura, Y., Makita, H., and Kawashima, Y., **J. Microencapsulation.**, 1993, 10, 461.

6. Kawashima, Y., Okumura, M., and Takenaka, H., **Science.**, 1982, 216, 1127.

7. Paradkar, A.R., Pawar, A.P., Deshpande, M.C. and Gat, G.V.," Spherical Crystallisation of Sulphamethoxazole", Paper presented at **International Seminar on Recent Advances in Pharmaceutical Sciences**; Ooty, Feb. 1995.

8. Kawashima, Y., Morishima, M., Takenchi, H., Niwa. T., and Hino. T., **AlchE Symp. Ser.**, 1991, 87, 26, (through Chem. Abstract 115: 2 63325k 1991).

9. Kawashima Y., Takenaka H., and Hino. T., **Chem. Pharm. Bull.**, 1990, 38, 2537.

10. Edwards, E.J., Gore, D.N., Rapson, H.D.C., and Taylor, M.P., **J. Pharm. Pharmacol.**, 1994, 7, 924.

Structural Education of Columbin, A Diterpene Isolated from The Rhizomes of *Artistolochia albida*

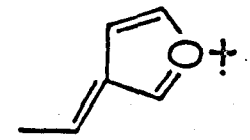
[1]M. K. CHOUDHURY*, [1]A. K. HARUNA, [1]E. C. JOHNSON AND [2]P. J. HOUGHTON
[1]Dept. of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria
[2]Dept. of Pharmacy, king's College, London
Manresa Road, Chelsea, London SW3 6LX, U.K.

Received 21st May 1996

The Isolation and structure elucidation of columbia have been reported from Aristolochia albida as well as from Aristolochiaceae family for the first time possessing antisnake venom activities, the structure of which was determined by special (UV, IR, ^{1}H-NMR, ^{13}C-NMR, MS) and elemental analysis. This is the first report of biological activities of Columbin.

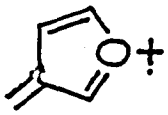
THE presence of sterol and D-glucose [1], the *in vivo* antisnake venom activities of a furanoid diterpene[2] isolated from the rhizomes of Aristolochia albida Duch (family : Aristolochiaceae) were previously reported from this laboratory. The present article describes the structure elucidation of this biologically active furanoid diterpene lactone which has been characterized as columbin on the basis

* For correspondence

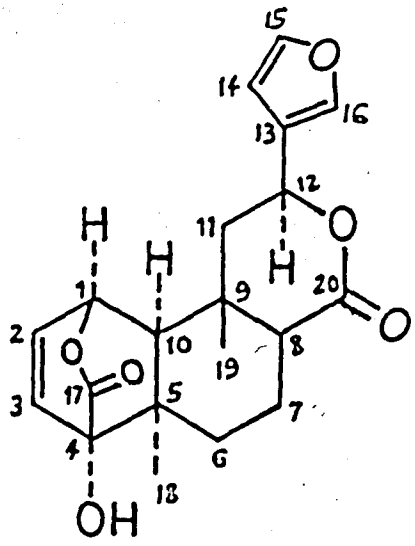


ion a, m/z 95

ion b, m/z 94



ion c, m/z 81



Columbin

of spectral and elemental analysis. The diterpene possesses antisnake venom activities against Naja nigricollis (spitting cobra) and Bitis arietans (puff-adder), the two snake species found in Northern Nigeria.

The occurrence of columbin was reported earlier from other sources like roots of Jateorrhiza palmata Miers (Menispermaceae)[3],seeds of Sphenocentrum jollyanum Pierre (Menispermaceae)[4], roots of Melothria maderospatana (Cucurbitaceae)[5] and seeds of Dioscoreophyllum cumminsii (Menispermaceae)[6] but nothing was mentioned about its biological activity.

The plant was collected in mid-July from a forest reserve in Katsina state, nigeria and authenticated by the Ahmadu Bello University herbarium, Zaria, Nigeria. Melting point was determined using Gallenkamp melting point appratus and is uncorrected. The UV spectrum was recorded on Pye Unicam 5000 Spectrometer SP 8-100. IR spectrum was measured on Perkin-Elmer 1710 FT Spectrophotometer. ^{1}H-NMR spectra were run on Bruker WM 250 instrument at 250 MHz. ^{13} C-NMR spectra were recorded on Bruker 250 13C Spectrometer.

The air dried powdered rhizomes after defatting with light petroleum (60-80°) were extracted with methnol (Soxhlet). Upon evaporate of the solvent, a copious white precipitate formed which on repeated crystallisation from chloroform/ methnol gave colourless needles,mp.182°. The formula was determined as $C_{20}H_{22}O_{26}$ on the basis of mass spectrum and C,H- analysis. It gave pink turning to violet colouration with Liebermann Burchard reagent (80 ml EtOH + 10 ml Ac_2O + 10 ml conc. H_2SO_4) for terpenoid.

UV (MeOH) : 204 nm (∈ 4,081)[7].

IR (Nujol) : 3503 (OH), 1746 (δ - lactone), 1703 (δ - lactone) and 3131, 1501, 909, 875 cm^{-1} (furan ring)[8-15].

GC-MS : RT 10.343 min. It did not show molecular ion peak M^+ at m/z 358. The predominant fragentations were at m/z 314 (48,M^+ - CO_2),296 (24,M^+-CO_2- H_2O), 222 (32, M^+ - $2CO_2$ - $2CH_3$), 204 (100), 203 (25, 204 - H), 113 (16), 109 (56),95 (48,ion a), 94 (60, ion b), 81 (36, ion c), 78 (56). The fragment ions at m/z 81 (corresponding to fission of C-11 / C-12 bond) and m/z 94 (fission of C-9/C-11 bond) indicated the presence of a furan ring in the usual position at C-12[13,15-18].

MS (Chemical ionization, CI positive) : m/z 359 (M^++ 1).

^{1}H-NMR (250 MHz, $CDCL_3$, TMS) δ,J in Hz : 1.05 (3H, s, CH_3 at C-9), 1.24 (3H, s, CH_3 at C- 5), 3.52

(1H, s, D_2O exchangeable, OH), 5.15 (1H, dd, $J_{1,2}$ = 5, $J_{1,3}$ = 2, H-1), 6.47 (1H, dd, $J_{2,1}$ = 5, $J_{2,3}$ = 8, H-2), 6.36 (1H, dd, $J_{3,2}$ = 8, $J_{3,1}$ = 2, H-3), 2.07 (1H, m, H-7 axial), 2.40 (1H, dd, J = 2, 11, H-6 equatorial), 1.40 (1H, m, H-7 axial), 1.78 (1H, dd, J = 1.5, 8, H-7 equatorial), 2.65 (1H, m, H-8), 1.95 (1H, dd, $J_{11a, 11b}$ = 15, $J_{11a,12}$ = 12, H-11a, axial), 2.27 (1H, dd, $J_{11a, 11b}$ = 15, $J_{11b,12}$ = 4.5, H-11b, equatorial), 5.42 (1H, dd, $J_{12,11a}$ = 12), $J_{12,11b}$ = 4.5, H-12), 6.45 (1H, dd, $J_{14,15}$ = 1.5, $J_{14,16}$ = 1, H-14), 7.44 (1H, dd, $J_{15,14}$ = 1.5, $J_{15,16}$ = 1.5, H-15) and 7.48 (1H, dd, $J_{16,15}$ = 1.5, $J_{16,14}$ = 1, H-16)[8,12,13,16]. The H-10 proton appeared as singlet at 1.75 and weakly coupled to H-1 proton.

^{13}C-NMR (62.5 MHz, $CDCl_3$, TMS, DEPT) δ : 74.18 (CH, C-1), 128.68 (CH, C-2), 136.84 (CH, C-3), 80.48 (C, C-4), 37.16 (C, C-5), 25.59 (CH_2, C- 6), 17.33 (CH_2, C-7), 47.58 (CH, C-8), 35.28 (C, C-9), 44.49 (CH, C-10), 41.90 (CH_2, C-11), 70.66 (CH, C-12), 124.79 (C, C-13), 108.40 (CH, C-14), 139.66 (CH, C-15), 143.96 (CH, C-16), 175.48 (CO, C-17), 172.37 (CO, C-18), 27.0 (CH_3, C-19), 24.31 (CH_3, C-20).

C,H-analysis : Found : C, 67.09; H, 5.98; $C_{20}H_{22}O_6$ requires C, 67.03; H, 6.19%.

R_f values (TLC) of the compound identified as columbin (precoated on plastic polygram SIL G, UV 254, 0.25 mm, Camlab, Germany) in different solvent systems obtained were, $CHCl_3$ = 0.11, $CHCl_3$ - MeOH (98:2) = 0.39, $CHCl_3$ - EtOAc (4:1) = 0.42, $CHCl_3$ - EtOAc (3:1) = 0.50, $CHCl_3$ - AtOAc (2:1) = 0.56, C_6H_6 - $CHCl_3$ - MeOH (6:3:1) = 0.57, EtOAc = 0.95.

In silica gel G, Merck, 0.25 mm; $CHCl_3$ (1:9) columbin, R_f = 0.44; isocolumbin, R_f = 0.36; C_6H_6 - $CHCl_3$ (1:9), columbin, R_f = 0.41; isocolumbin, R_f = 0.35.

The easy epimerization of columbin to isocolumbin was performed by mild treatment of the compound with dilute sodium hydroxide which was detected as a very close more polar spot than columbin on TLC. The possibility for the presence of isocolumbin in the plant was ruled out by TLC examination of the methanolic extract of the rhizomes when a spot corresponding to columbin was detected only, no spot corresponding to isocolumbin (more polar) was observed.

The diterpene was characterized as columbin on the basis of comparative studies and observations made so far. From the view point of phytochemistry, it deserved special mention that the occurrence of columbin has been observed for the first time in **Aristolochia** species and also in the family Aristolochiaceae. The pharmacological importance of columbin was not evaluated before.

ACKNOWLEDGEMENT

The authors express their sincere thanks and gratitude to Dr. (Mrs.) Meenakshi Choudhury for her kind assistance during the work.

REFERENCES

1. Choudhury, M.K. and Haruna,A.K., **Indian J. Pharm. Sci.**, 1994, 56, 230.
2. Haruna, A.K. and Choudhury, M.K., **Indian J. Pharm. Sci.**, 1995, 57, 222.
3. Wessely F., Dinjaski, K., Isemann, W. and Singer, G., **Monatsh.**, 1935, 66, 87.
4. Gilbert, J.N.T., Mathieson, D.W. and Patel, M.B., **Phytochemistry.**, 1967, 6, 135.
5. Chen, Y.P., Hsu, H.Y., Ruo, T.T., Iguchi, K. and Kakisawa, H., **Phytochemistry.**, 1973, 12, 3000.
6. Ramstad, E., Powell, J.W., Wilson, B.J., Adesina, S.K., Higginbotham, J.D. and Harborne, J.B., **Phytochemistry.**, 1975, 14, 2719.
7. Barton, D.H.R. and Elad, D., **J. Chem. Soc.**, 1956, 2085.
8. Eagle, G.A. and Rivett, D.E.A., **J. Chem. Soc. Parkin Trans 1.**, 1973, 1701.
9. Hori, T., Kiang, A.K., Nakanishi, K., Sasaki, S. and Woods, M.C., **Tetrahedron.**, 1967, 23, 2649.

10. Ito, K. and Furukawa, H., **J. Chem. Soc. Chem. Commun.**, 1969, 653.

11. Anthonsen, T., McCabe, P.H., Crindle, R.M. and Murray, R.D.H., **Tetrahedron.**, 1969, 25, 2233.

12. Yonemitsu, M., Fukuda, N., Kimura, T. and Komori, T., **Liebigs Ann. Chem.**, 1986, 1327.

13. Fukuda, N., Yonemitsu, M. and Kimura, T., **Chem. Pharm. Bull.**, 1986, 34, 2868.

14. Savona, G., Bruno, M., Paternostro, M., Marco, J.L. and Rodriguez, B., **Phytochemistry.**, 1982, 21, 2563.

15. Rahman, A.U. and Ahmad, S., **Phytochemistry**, 1988, 27, 1882.

16. Anthonsen, T., McCabe, P.H., McCrindle, R. and Murray, R.D.H., **Chem. Commun.**, 1966, 740.

17. Budzikiewicz, H., Djerassi, C. and Williams, D.H.; Structure Elucidation of Natural Products by Mass Spectrometry, Vol. II Holden-Day, San Francisco, 1964, 135.

18. Ahmad, M., Khaleque, A. and Wahed Mian, M.A., **Indian J. Chem.**, 1978, 16B, 317.

Determination of acetaminophen in presence of codeine in pharmaceutical formulations by derivative spectrophotometry

J. HANAEE,
Dept. of Pharmaceutical Chemistry, Medical Sciences, University of Tabriz, Tabriz, IRAN.

Received 21 May 1996

First derivative U.V. spectrophotometry has been used for the assay of acetaminophen in presence of codeine. Acetaminophen has been assayed by measuring the first derivative absorbances at 263.4 nm. The concentration of acetaminophen has been calculated without interference of codeine. The procedure is simple and rapid, and provides accurate and precise results.

ACETAMINOPHEN-CODEINE tablets are widely used as analgesic antipyretics. Several methods have been published for the determination of acetaminophen in pharmaceutical formulations, alone or in presence of other components. They include colorimetric, titrimetric, HPLC, GLC and orthogonal function methods[1-6]. All these methods are, however, time consuming and require sophisticated equipments. Therefore, the purpose of the present investigation is to develop a rapid and simple U.V. first derivative spectrophotometric method for the determination of acetaminophen in presence of codeine in pharmaceutical formulations which can be easily adopted in a drug control laboratory as well as pharmaceutical industry.

Pure acetaminophen powder and codeine phosphate were purchased from Merck company and acetaminophen-codeine tablets from Iranian Daroupakhsh pharmaceutical company. Acetaminophen stock solution (50 mg/l) was prepared in 96% ethanol. The first derivative U.V. spectra of working standard solutions, containing 10-20 mg/l of acetaminophen were recorded over the 200-400 nm range against solvent blank and the absorbances at 263.4 nm were measured using Shimadzu double beam spectrophotometer. Accurately weighed amounts of pure acetaminophen with increasing amounts of pure codeine were dissolved in the ethanol. Acetaminophen concentration was obtained by interpolating the calibration curve (Table 1). Also the