Phytochemistry, Vol 28, No. 10, pp 2759–2761, 1989
Printed in Great Britain

0031–9422/89 $3 00 + 0 00
© 1989 Pergamon Press plc

A CLERODANE DITERPENE AND OTHER CONSTITUENTS OF *CROTON MEGALOCARPUS**

IVAN ADDAE-MENSAH, REINER WAIBEL,† HANS ACHENBACH,† GICHURU MURIUKI, CLIVE PEARCE‡ and JEREMY K. M SANDERS‡

Department of Pharmacy, University of Nairobi, Kenya; †Department of Pharmaceutical Chemistry, Institute of Pharmacy, University of Erlangen, F R G., ‡University Chemical Laboratories, Lensfield Road, Cambridge, U K

(*Received* 20 *December* 1988)

Key Word Index—*Croton megalocarpus*; Euphorbiaceae, diterpene, clerodane, chiromodine; lupeol, β-sitosterol; betulin; long chain aliphatic esters

Abstract—A novel clerodane-type furano-diterpene with the 9,10-abeo-methyl group has been isolated from *Croton megalocarpus* as a major constituent. Structure elucidation has been achieved by a combination of spectroscopic measurements including 2D-NMR and NOE experiments, on the parent compound as well as the monoacetyl derivative. Lupeol, betulin, β-sitosterol and long-chain aliphatic esters were also found and characterized

INTRODUCTION

Various *Croton* species have yielded a number of diterpenes which have been found to possess anti-ulcer [2], anticancer [3], and co-carcinogenic properties [4]. As part of our studies of plants from the genus *Croton* growing in East Africa, which have hitherto received virtually no phytochemical or pharmacological examination, we have investigated the constituents of *Croton megalocarpus* (local name: Musine). As a major component, we isolated a novel furano-diterpene belonging to the rare clerodane-type compounds, which possess a 9,10-abeo-methyl group [5] We have named it chiromodine (**1**).

RESULTS AND DISCUSSION

The petrol extract gave a white crystalline compound, mp 205–206°, whose IR spectrum shows OH absorption at ν_{max} 3470 cm^{-1}. Peaks at 3100, 1515 and 875 cm^{-1}, as well as a UV absorption maximum at λ_{max} 252 nm and a positive Ehrlich test, suggested the presence of a furan ring system [6]. The ^1H NMR spectrum is consistent with a β-substituted furan ring and this is further supported by the presence of the base peak at *m/z* 95 ($C_5H_3O_2$) in the MS, arising from a furanyl-carbonyl group. The ^1H NMR spectrum demonstrates the presence of three methyl groups (δ1.24, 1.55 and 1.59) at quarternary carbons, as well as a methoxyl group (δ3.59). A ^{13}C signal at δ175 5 has to be attributed to an ester carbonyl. Strong peaks in the MS at *m/z* 268 and 110 and in particular evidence from ^1H and ^{13}C NMR suggest a clerodane-type ring system [7], to which a β-keto-substituted-furan and a methoxycarbonyl group are attached. From the Me-resonance in the ^{13}C NMR at δ17 73, attributed to Me-19 (^1H: δ1.24, *s*, 3H), it can be deduced that this methyl group (at C-5) stands at a *trans*-fused ring-system, since *cis*-fused rings should shift the ^{13}C NMR signal of a methyl (at C-5) characteristically to the low-field of *ca* δ33 [8, 9]. A ^1H homonuclear COSY-experiment revealed partial structures **A** and **B**.

Heteronuclear 2D-studies ($^1H/^{13}C$ COSY, $^1H/^{13}C$ COLOC) allowed the combination of **A** and **B**, and in addition located the furanyl-carbonyl and the methoxycarbonyl group as depicted in formula **2** The most important $^1H/^{13}C$ couplings are shown in **3**. ^1H NMR and ^{13}C NMR assignments are summarized in Table 1.

Our values are in good agreement with published NMR resonance data in the clerodane series [7] Differential NOE experiments on the parent compound and its monoacetyl derivative (**1a**) established the relative configuration **1** In addition to chiromodine (**1**), lupeol, β-sitosterol, betulin and a mixture of long-chain aliphatic fatty esters [among them $C_{21}H_{43}COO(CH_2)_nMe$, $C_{23}H_{47}COO(CH_2)_nMe$ and $C_{25}H_{51}COO(CH_2)_nMe$ (n = 19, 21)] were also isolated from the petrol extract.

EXPERIMENTAL

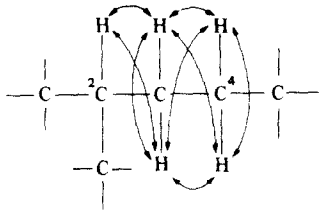
Plant material was collected in October and November 1986 from the Chiromo campus of the University of Nairobi A voucher specimen is deposited at the Pharmacy Department, University of Nairobi under No CROT/86/2

IR KBr, UV MeOH, NMR, 400 MHz, C_5D_5N or $CDCl_3$, TMS as int standard

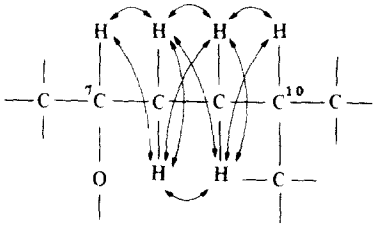
Extraction and isolation. The powdered stem bark (2 kg) was exhaustively extracted for 48 hr with petrol (bp 60–80°), after evapn of the solvent the oily residue was kept at −20° for 48 hr Trituration of the cold residue with Et_2O led to the instantaneous precipitation of the title compound.

Chiromodine (**1**). Repeated recrystallization from MeOH gave white needles (0 439 g), mp 205–206°; $[\alpha]_D^{20} = -15°$ (MeOH; *c* 0 39), UV λ_{max} 215sh (log ε 3 7) and 252 nm (log ε 3.5); IR ν_{max} cm^{-1}

*Part 37 in the series 'Constituents of Tropical Medicinal Plants' For part 36 see [1]



A



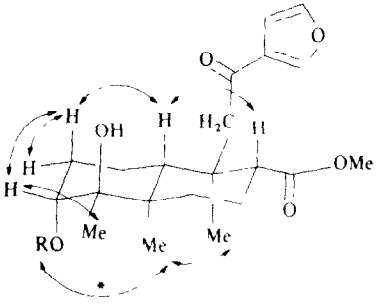
B

3400 (OH), 1710 (ester C=O), 1660 (α,β-unsatd C=O) MS *m/z* (rel int ≧ 15%) 378 (16) $[M]^+$, 269 (16) 268 (76) 250 (15) 195 (58), 177 (19), 173 (54), 163 (20), 147 (29), 137 (16), 135 (34) 122 (18), 121 (28), 119 (18), 110 (23), 107 (20), 95 (100), 43 (18) ^1H and ^{13}C NMR see Table 1

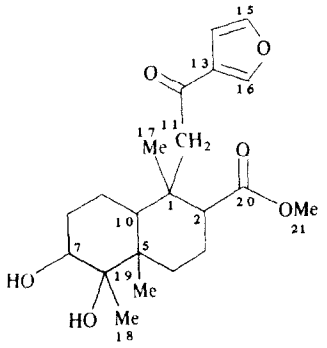
Chiromodine acetate (**1a**) 10 mg **1** were dissolved in 1 ml Ac_2O–pyridine (1 1) and stirred at room temp (48 hr) After evapn of the solvent, the crude product was purified on 3 g silica gel using hexane–EtOAc (4 1) as eluent This yielded white needles (7 8 mg), mp 178–180°, $[\alpha]_D^{20} = -40°$ ($CHCl_3$, *c* 1 3), MS *m/z* (rel int. ≧ 10%) 420 (4) $[M]^+$, 250 (10), 232 (10), 194 (15), 173 (36), 121 (13), 110 (16), 95 (89), 43 (100), ^1H NMR ($CDCl_3$) δ 8 02 (1H, *br s*), 7 41 (1H, *dd*, $J_1 \sim J_2 \sim 1$ 5 Hz) 6 76 (1H, *dd* $J_1 = 1$ 5, $J_2 = 0$ 5 Hz), 4 71 (1H, *dd*, $J_1 \sim J_2 \sim 3$ Hz), 3 60 (3H, *s*), 3 16 (1H, *dd*, $J_1 = 12.5$, $J_2 = 3$ 5 Hz), 2 93 (1H, *d*, $J = 17$ Hz), 2 86 (1H, *d*, $J = 17$ Hz), 2 58 (1H, *dd*, $J_1 = 12$ $J_2 = 2$ Hz), 2 07 (3H, *s*), 2 00–1 84 (2H), 1 79–1 35 (6H), 1 15 (3H, *s*) 1 12 (3H, *s*) 1 00 (3H, *s*)

Other constituents. The Et_2O mother liquor was evapd and triturated with MeOH Repeated chromatography of the MeOH-insoluble part (6 7 g) on silica gel with petrol–$CHCl_3$ (1 1) and C_6H_6–petrol (1 1) gave some more chiromodine (125 mg) and a wax-like fraction (105 mg), which appeared homogeneous on TLC but according to spectral data in particular, was found to be a mixture of esters of $C_{21}H_{43}CO_2H$, $C_{23}H_{47}CO_2H$ and $C_{25}H_{51}CO_2H$ with alkanols in the C_{20}–/C_{22}-range

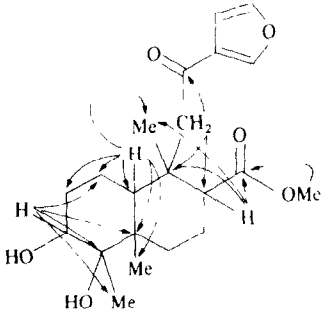
Chromatography of the MeOH-soluble part (82 g) on silica gel with benzene and then solvents of increasing polarity yielded lupeol (3 0 g), betulin (0 05 g) and β-sitosterol (0 02 g)



1 R = H (* NOE observed in **1a** only)
1a R = Ac



2



3

Table 1 ^{1}H NMR and ^{13}C NMR data of **1**

H	δ	J (Hz)	C	δ
2_{ax}	3.65 *dd*	13.0, 3.5	1	40.87
3_{ax}	2.19 *dddd*	13.0, 13.0, 13.0, 3.0	2	50 07
3_{eq}	1.77 *dddd*	13.0, 3 0, 3.0, 3.0	3	21.78
4_{ax}	2 27 *ddd*	13.0, 13.0, 3.0	4	32.06
4_{eq}	1.60 *ddd*	13.0, 3.0, 3.0	5	42.28*
7_{eq}	4.03 *dd*	3.0, 3.0	6	76 04
8_{ax}	2.40 *dddd*	13 0, 13.0, 3.0, 3.0	7	76.47
8_{eq}	1.93 *dddd*	13.0, 3.0, 3.0, 3.0	8	31.52
9_{ax}	2.07 *dddd*	13.0, 13.0, 13.0, 3.0	9	18 42
9_{eq}	1.66 *br d*	13.0	10	42.35*
10_{ax}	2.99 *dd*	12.5, 2.0	11	49.09
11	3 11 *d*	16.5	12	194.66
	3.27 *d*	16.5	13	130 13
14	6.92 *br d*	2.0	14	109.33
15	7.56 *dd*	2.0, 2.0	15	144.66
16	8.49 *br*		16	148.15
Me-17	1 24 *s*		17	19.72
Me-18	1.59 *s*		18	22.04
Me-19	1.55 *s*		19	17.73
Me-21	3.59 *s*		20	175.51
			21	50.91

*Assignments may be interchanged.

Acknowledgements—Thanks are due to the University of Nairobi, the Alexander von Humboldt Foundation and the German Foundation for International Development (DSE) for very generous support. We also thank the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie for financial support.

REFERENCES

1. Torrenegra, R., Bauereiß, P. and Achenbach, H. (1989) *Phytochemistry* **28**, 2219.
2. Kitazawa, E., Sato, A., Takahashi, S., Kuwano, H. and Ogiso, A. (1980) *Chem. Pharm. Bull.* **28**, 227.
3. Kupchan, S. M., Uchida, I., Branfman, A. R., Dailey, R. G., Jr and Fei, B. Yu (1976) *Science* **191**, 571.
4. Weber, J. and Hecker, E. (1978) *Experientia* **34**, 679.
5. Claude-Lafontaine, A., Rouillard, M., Cassan, J and Azzaro, M. (1976) *Bull. Soc. Chim. Fr.* 88.
6. Chatterjee, A., Banerjee, A. and Bohlmann, F (1977) *Tetrahedron* **33**, 2407.
7. Wehrli, F. W. and Nishida, T. (1979) in *Fortschritte der Chemie Organischer Naturstoffe*, (Herz, W., Grisebach, H., Kirby, G. W., ed.). Vol. 36, p. 1. Springer, Wien.
8. Niwa, M., Kihira, N., Hirata, Y., Tori, M., Wu, T. S. and Kuoh, C. S. (1987) *Phytochemistry* **26**, 3293.
9. De Rosa, S., Minale, L., Riccio, R. and Sodano, G (1976) *J. Chem Soc. Perkin Trans. I* 1408