11/71 respectively, deposited at Jipmer) *Uses.* Medicinal [1] *Previous work* Flavonoids of leaves [2-4]

Present work A systematic examination of the C_6H_6 extractives of the leaves of all the above *Clerodendron* species revealed the presence of a sterol, this was separated by adsorption chromatography over neutral Al_2O_3 using light petrol, C_6H_6 and $CHCl_3$ in different proportions successively, the C_6H_6 eluate yielded colourless needles, $C_{29}H_{46}O$, m p 151–153°. Its acetate, m p 147–148°, had the following spectral characteristics ν_{KBr}^{max} 1728 (ester), 1640 and 882 (=CH_2) and 957 cm^{-1} (trans disubstituted double bond) NMR (δ values) 0 70 (*s*, 3H, $C_{18} \rightarrow$ Me), 0 83 (*t*, *J* 7 Hz, 3H, $-CH_2-C\underline{H}_3$) 0 99 (*d*, *J* 6 Hz, 3H, $-CH-C\underline{H}_3$), 1 03 (*s*, 3H, $C_{19} \rightarrow$ Me), 1 63 (*s*, 3H, $C_{27} \rightarrow$ Me), 2 02 (*s*, 3H, –O–CO–Me), 4·03 (*m*, 1H, C_3–H), 4 69 (*s*, *br*, 2H, $>C=CH_2$), 5 23 (*m*, 2H, –CH=CH) and 5 40 (*m*, 1H, C_6–H) The parent compound was identified as (24*S*)-ethylcholesta-5,22,25-triene-3β-ol and the identity was confirmed by direct comparison, m m p and co-TLC ($AgNO_3$ impregnated silica gel) with an authentic specimen

Comment The title compound was isolated for the first time as a natural product by Bolger *et al* from *Clerodendrum campbellii*[5] and subsequently by Joshi and Kamat from *Enhydra fluctuans* (Compositae) [6] The present report of its occurrence from four more *Clerodendron* species suggests that this sterol may be considered as a possible chemotaxonomic marker of the genus *Clerodendron*

Acknowledgements—We thank Professor T R Govindachari for the spectral data, Dr B S Joshi for an authentic specimen of the sterol and the Principal, J I P M E R, for encouragement.

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ZYGACINE AND ZYGADENINE: THE MAJOR ALKALOIDS FROM *ZYGADENUS GRAMINEUS*

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(*Received* 27 *February* 1973 *Accepted* 16 *March* 1973)

Key Word Index—*Zygadenus gramineus*, Liliaceae, ceveratrum alkaloids, zygacine, zygadenine

Plant Zygadenus gramineus–Liliaceae *Source* Antelope Range Experimental Station, Buffalo, South Dakota (voucher specimen is deposited in the College of Pharmacy).

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Marsh and Clawson[1] studied the toxicity of five species of Zygadenus and showed that *Zygadenus gramineus* was the most toxic Reid and Smith[2] were able to isolate one alkaloid, zygadenine However, Reid and Phillips[3] were able to show by PC that the plant contained at least four alkaloids The major alkaloid was less polar than zygadenine

Whole, frozen and thawed, *Zygadenus gramineus* plants (366 g) were ground in a blender with $CHCl_3$ (1000 ml) and NH_4OH (250 ml) After grinding, $CHCl_3$ (1500 ml) was added and the mixture stirred for 3 days, filtered through cheese cloth and the $CHCl_3$ separated The $CHCl_3$ was evaporated to 75 ml, and extracted with N HCl (5 × 100 ml) The HCl solution was cooled in ice, made alkaline with NH_4OH, and extracted with $CHCl_3$ The $CHCl_3$ solution was dried (Na_2SO_4) and evaporated to yield 0 123 g of crude alkaloids

The crude alkaloids were examined by TLC. The alkaloids were separated on silica gel-GF-254 using solvent 1, C_6H_6–EtOAc–diethylamine (7 2 1) and/or solvent 2, $CHCl_3$–MeOH–NH_4OH (8 2 1) The alkaloids were located by spraying with H_2SO_4–MeOH, 1 1 or basic $KMnO_4$ The R_fs in solvent 1 were 0 09 (zygadenine), 0 56 (zygacine), 0 71, and 0 82 The R_fs in solvent 2 were 0 22, 0 47 (zygadenine), 0 64, 0 74 (zygacine), and 0 87 When the alkaloids were examined by 2-D TLC, in the same solvents, they separated into 10 components In every system, the alkaloid whose R_f was identical to zygacine was the most abundant and that whose R_f was identical to zygadenine was second

The two major alkaloids were separated by preparative TLC using solvent 1 Zygadenine was eluted from the silica gel by $CHCl_3$–MeOH–NH_4OH (1 1 0 02) It was precipitated from acetone by Et_2O and then recrystallized from acetone It was identified by m p 215–220° (lit 218–220°)[4] and IR (KBr) which was identical with an authentic sample

Zygacine was eluted with $CHCl_3$–NH_4OH (1 0 02). It was recrystallized from acetone hexane m p 198–202° The identity was confirmed by IR(KBr) 1740 and 1250 cm^{-1} (characteristic of 3-acetosteroids[5]), MS (70 eV) (rel intensity) 535 (9), 475 (14), 112 (999), and 43 (82) (in agreement with that obtained by Budzikiewicz)[6]

Acknowledgements—The author thanks Dr S M Kupchan for providing samples of several ceveratrum alkaloids, W R Trevillyan for locating and identifying the plants, and the National Institutes of Health for support through Research Grant 1 RO1 HE 13613 MCHA

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