was filtered and then freeze-dried. The residue was treated with EtOH to yield **2** (6 5 mg) as colourless needles (undepressed mixed melting and superimposable IR spectra with authentic **2**. The sugar was reconfirmed as D-glucose by the procedure as described for acid hydrolysis

Acknowledgements—The authors thank Prof. U Sankawa for the high resolution NMR spectra. One of the authors (KLC) wishes to express his gratitude to UNESCO for the Exchange of Scientists Scheme Award and to Science University of Malaysia for a study leave at the Natural Products Research Institute in Seoul

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Phytochemistry, Vol 28, No 10, pp 2859–2861, 1989
Printed in Great Britain

0031–9422/89 $3 00+0 00

A STEROID FROM *CALOTROPIS PROCERA*

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(*Received* 24 *January* 1989)

Key Word Index—*Calotropis procera*, Asclepiadaceae; procesterol; hydroxyketone, (24*S*)-24-ethyl stigmast-4-en-6α-ol-3-one

Abstract—Procesterol, a new steroidal hydroxy ketone, has been isolated from the fresh and undried flowers of *Calotropis procera*. The chemical and spectral studies identified it as a C-6, C-24 diepimer of stigmast-4-en-6β-ol-3-one.

INTRODUCTION

Calotropis procera R. Br. (Asclepiadaceae) grows widely in tropical regions of Asia and Africa. The milky juice of this plant is used by the natives of India as a purgative, while the flowers are considered digestive, stomachic, tonic and useful in cough, asthma, catarrh and loss of appetite. The root bark is said to promote secretions and to be useful in treating skin diseases, enlargement of the abdominal viscera, intestinal worms, ascites and anasarca [1]. We have previously reported a new terpenoid from this plant [2] Following further studies on the fresh and undried flowers a new keto-sterol has been isolated and named as procesterol Its stereostructure has been elucidated as (24*S*)-24-ethyl-stigmast-4-en-6α-ol-3-one, through chemical and spectral studies

RESULTS AND DISCUSSION

Procesterol (**1**) crystallized from acetone, mp,. 167°, $[\alpha]_D+21.5°$; its HRMS gave a molecular ion peak at *m/z* 428.6654 corresponding to the molecular formula $C_{29}H_{48}O_2$ (calcd 428.7010) The molecular ion peak was also confirmed by FD mass spectrometry [3]. The strong absorption band at 1675 and 1640 cm^{-1} in its IR spectrum revealed the presence of an α,β-unsaturated keto function and another band at 3575 cm^{-1} was due to hydroxyl absorption. The secondary nature of alcoholic group in **1** was shown by its oxidation with Jones reagent to a diketone **1a**. The ^1H NMR spectrum showed the presence of 6-methyl groups out of which one was primary [δ0.847, 3H, *t*, $J=7.3$ Hz], three secondary [δ0.905, 0.827, 0.847, 3×3H, *d*, $J=6.3$, 6 7 Hz] and two tertiary [δ1.397, 0 760, 2×3H, *s*]. Further signals were observed for vinylic proton [δ5.780, 1H, *d*, $J=1.8$ Hz] and the proton geminal to hydroxyl group [δ4.450, 1H, *oct.*, $J=12$ 1, 4.7, 1.8 Hz]. The ^{13}C NMR spectrum revealed 29 carbon atoms in the molecule. The multiplicity assignments were made by DEPT experiments [4] which showed the presence of six methyl, 10 methylene and nine methine carbon atoms. The quaternary carbon atoms were determined by subtracting DEPT spectra from broad band ^{13}C NMR spectrum.

Procesterol showed in its mass spectrum prominent [M − side chain]$^+$ (*m/z* 287), [M − side chain − 42]$^+$ (*m/z*

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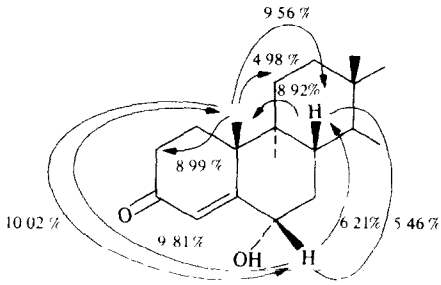

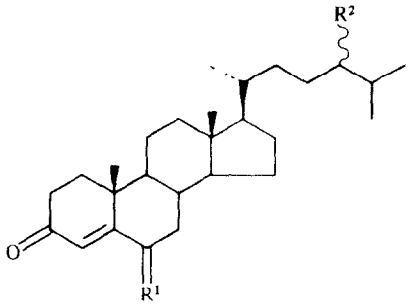

1 R^1 = αOH, βH R^2 = $(S)C_2H_5$
1a R^1 = O, R^2 = $(S)C_2H_5$
1b R^1 = βOH, αH, R^2 = $(R)C_2H_5$

245), and $[M-\text{side chain}-42-H_2O]^+$ (*m/z* 227) The peaks at *m/z* 245 and 227, respectively, and a significant peak at *m/z* 152 $[M-\text{side chain}-42-93]^+$ were characteristic of 3-keto-6-hydroxy-Δ^4 steroid [5]

The mass spectrum revealed the presence of an ethyl group in the side chain and position 24 was assigned to it on the basis of biogenetic analogy as well as close similarities in chemical shifts of the protons and carbons of the side chain with related compounds Procesterol has, therefore, the same basic skeleton as stigmast-4-en-6β-ol-3-one (**1b**) In so far, however, as the two compounds and their oxidation products differ widely in melting point and optical rotation, the former is evidently an epimer of latter.

The assignments of ^{13}C NMR were made by comparing the values with the published ^{13}C NMR data of related sterols [6, 7] and confirmed by ^1H–^{13}C heteronuclear chemical shift correlated spectrum (hetero COSY) [8]. The coupling interactions were established through the 2D homonuclear ^1H–^1H chemical shift correlation measurements (COSY-45°), while the multiplicity of the overlapping proton signals was determined from the 2D *J*-resolved spectrum

The coupling interaction of 6-H revealed that the hydroxyl group on this position has α- and equatorial orientation unlike β- and axial orientation of the same group in **1b** In **1** the 6-βH appeared as an octate showing axial–axial coupling with 7-αH ($J = 12.1$ Hz), axial–equatorial coupling with 7-βH ($J = 4.7$ Hz) and an allylic coupling with 4-H ($J = 1.8$ Hz) On the contrary, the different coupling interactions, particularly the absence of coupling between 6α-H and 4-H, are characteristic for compounds of the type **1b** being rationalized by significant change in dihedral angles [9]. The coupling interactions of 6-βH were also illustrated by COSY-45° which showed strong cross peaks of this proton with those of 4-H at δ5 780, 7-αH at δ1.310 and 7-βH at δ1 342. Conclusive evidence for α- and equatorial orientation of hydroxyl group was provided by NOE difference measurements Irradiation at δ4.450 (6-βH) resulted in a 9 81% NOE at δ1 397 (19-H_3) and 6.21% NOE at δ1.420 (8-βH). The irradiation at δ1.397 (19-H_3) resulted in a 10 02% NOE at δ4 450 (6-βH), 9 56% NOE at δ1 420 (8-βH), 8.99% NOE at δ1 980 (2-H_2), and 4 98% NOE at δ1 29 (11-H_2) Finally irradiation at δ1 420 (8-βH) resulted in a 5 40% at δ4 45 (6-βH) and 8.92% NOE at δ1 397 (19-H_3)

The chemical shifts and coupling constants of various methyl groups in the side chain of **1** (except that of 21-methyl group) showed slight variation from **1b** indicating difference in stereochemistry of ethyl group at C-24 The *S* configuration in **1** was confirmed by comparison of chemical shifts of carbons and protons in ^{13}C and ^1H NMR spectra of **1** with a series of sterols having similar configuration at C-24, particularly (24*S*)-24-ethyl cholest-5-en-3β-ol and 5α-poriferastane [7, 10] The absolute structure of procesterol and its oxidation product could, therefore, be assigned To the best of our knowledge this is the first phytochemical report of steroidal hydroxy ketone epimeric at C-6 and C-24, and its isolation may be of chemotaxonomic significance

EXPERIMENTAL

General Mps uncorr ^1H and ^{13}C NMR TMS as int ref The DEPT experiments were carried out with θ45°, 90° and 135°, the quaternary carbons were determined by substration of these spectra from the broad band ^{13}C NMR spectrum For NOE measurement, the sample was frozen under liquid N_2 and degassed A lower decoupler power of maximum 0 2 W with 35 attenuation in dbs was used The pre-irradiation time was 1 1 sec which is the sum of three delays as used in the NOE difference programme of Bruker The impulse length of 10 μsec was maintained to avoid saturation The 2D COSY-45° data was acquired at 300 MHz with a sweep width of 4000 Hz (2K data points in ω_2) and 2000 Hz (256t_1 values zero-filled to 1K) in ω_1 The heteronuclear 2D ^1H–^{13}C chemical shift correlation experiments were carried out at 300 MHz with a sweep width of 12820 Hz (2K data point in ω_2) and 1 024 Hz (256t_1 values zero-filled to 2K) in ω_1 In both 2D experiments a 2 sec relaxation delay was used and 16 transients were performed for each t_1 value

Plant material The flowers of *C procera* were collected from the Karachi region and identified by the plant taxonomist, Department of Botany, University of Karachi, where a voucher specimen has been deposited

Extraction and isolation. The freshly collected plant material (20 kg) was extracted with EtOH at room temp The gummy residue obtained from the EtOH extract was partitioned between $CHCl_3$ and H_2O The hexane soluble portion of the $CHCl_3$ fraction was column chromatographed over silica gel The column was eluted with various solvent gradients of increasing polarities The fractions eluted with hexane–$CHCl_3$ (3 2) was evapd under red pres and again subjected to flash CC on silica gel Elution was carried out with gradients of increasing polarity using a mixture of hexane–Et_2O The eluate obtained from hexane–Et_2O (9·11) yielded a gummy residue which was finally purified by repeated crystallization from Me_2CO to afford procesterol (**1**) (yield 87 mg), mp 167 , $[\alpha]_D$ +21 5° ($CHCl_3$, *c* 0 21 7), IR $\nu_{max}^{CHCl_3}$ 3575 (OH), 1675 (C=O), 1640 (C=C) and 880

cm^{-1} (=CH). HRMS: M^+ 428.6654 ($C_{29}H_{48}O_2$); EIMS. *m/z* (rel. int.) 428 $[C_{29}H_{48}O_2]^+$ (15), 413 $[C_{29}H_{48}O_2-Me]^+$ (23), 410 $[C_{29}H_{48}O_2-H_2O]^+$ (18), 287 $[C_{29}H_{48}O_2-C_{10}H_{21}$ (entire substituent at C-17)]$^+$ (32), 269 $[C_{29}H_{48}O_2-C_{10}H_{21}-H_2O]^+$ (25), 245 $[C_{29}H_{48}O_2-C_{10}H_{21}-42]^+$ (43), 227 $[C_{29}H_{48}O_2-C_{10}H_{21}-42-H_2O]^+$ (45), 152 $[C_{29}H_{48}O_2-C_{10}H_{21}-42-93]^+$ (100), ^{1}H NMR: ($CDCl_3$) δ5 780 (*d*, *J* = 1.8 Hz, 4-H), 4.450 (*oct.* $J_{7\alpha,6\beta}$ = 12.1 Hz, $J_{7\beta,6\beta}$ = 4.7 Hz, $J_{6\beta,4}$ = 1.8 Hz, 6β-H), 1 379 (*s*, 19-H_3), 0 905 (*d*, *J* = 6.3 Hz, 21-H_3), 0 847 (*t*, *J* = 7.3 Hz, 29-H_3), 0 827 (*d*, *J* = 6.7 Hz, 27-H_3), 0.803 (*d*, *J* = 6.7 Hz, 26-H), 0 760 (*s*, 18-H); ^{13}C NMR ($CDCl_3$) δ38.66 (C-1), 34.31 (C-2), 200.30 (C-3), 126 59 (C-4), 168.43 (C-5), 73.36 (C-6), 37.17 (C-7), 45.93 (C-8), 53.72 (C-9), 38.08 (C-10), 21.04 (C-11), 39.68 (C-12), 42 59 (C-13), 56.79 (C-14), 24.32 (C-15), 28.22 (C-16), 56.16 (C-17), 11.92 (C-18), 19.84 (C-19), 36 28 (C-20), 18.78 (C-21), 33.99 (C-22), 26.43 (C-23), 46.11 (C-24), 29.01 (C-25), 19.09 (C-26), 19.59 (C-27), 23.16 (C-28), 12.30 (C-29).

Oxidation of procesterol. Procesterol (**1**) (20 mg) was dissolved in Me_2CO (40 ml) and treated with Jones reagent (6 ml). The reaction mixture with stirred at room temp. till the reaction was completed (TLC monitoring). Usual work-up and repeated crystallization from C_6H_6–HOAc (1:1) provided **1a**. (9 87 mg), mp. 98°; $[\alpha]_D$ + 72°, ($CDCl_3$, *c* 0.17), IR ν_{max} 1680 (C=O), 1605 (C=C), and 860 cm^{-1} (=CH); HRMS: M^+ 426.3511 ($C_{29}H_{46}O_2$); ^{1}H NMR ($CDCl_3$) δ6 098 (*s*, 4-H), 1.18 (*s*, 19-H_3), 0 906 (*d*, *J* = 6.3 Hz, 21-H_3), 0.847 (*t*, *J* = 7 3 Hz, 29-H_3), 0.827 (*d*, *J* = 6.7 Hz, 27-H_3), 0.804 (*d*, *J* = 6.7 Hz, 26-H_3), 0.771 (*s*, 18-H_3).

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Phytochemistry, Vol 28, No 10, pp 2861–2863, 1989
Printed in Great Britain

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

FORMATION OF THE *ORTHO*-QUINONE MANSONONE C FROM 7-HYDROXYCADALENE ON SILICA GEL

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(*Received in revised form* 24 *April* 1989)

Key Word Index—*Mansonia altissima*, Sterculiaceae, *Ulmus americana*; Ulmaceae; mansonone C; *ortho*-quinone; 7-hydroxycadalene; oxidation, silica gel.

Abstract—The sesquiterpene β-naphthol, 7-hydroxycadalene, found *inter alia* in the heartwood of a number of *Ulmus* spp., undergoes oxidation on silica gel to mansonone C, one of several related sesquiterpene *ortho*-quinones produced in some *Ulmus* spp. in response to certain stresses. The reaction, which appears to involve oxygen adsorbed on the silica gel, proceeds under an argon atmosphere and is dramatically retarded in the absence of light.

INTRODUCTION

Mansonones, a group of related sesquiterpene *ortho*-quinones originally isolated from the West African tree *Mansonia altissima* Chev. [1–4], were observed to accumulate in the sapwood of *Ulmus americana* and other elm species in response to infection by *Ceratocystis ulmi*, or other stresses [5–8]. Some of these compounds, including mansonone C, **1**, as well as possible biosynthetic precursors such as 7-hydroxycadalene, **2**, occur constitutively in the heartwood of several *Ulmus* species [9–11].

Mansonones C and E (**3**) have been prepared synthetically [9, 12–14], the former independently in two

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