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ISOLATION OF TWO SESQUITERPENES FROM *PLUCHEA ARGUTA*

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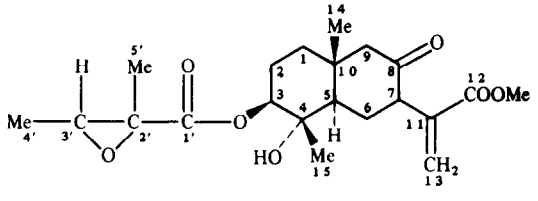
Abstract—Two new sesquiterpenes plucheicinin and 11,12,13-trinor-3,4-diepicuauhtemone have been obtained from the hexane- and ethyl acetate-soluble portions of the whole plant of *Pluchea arguta*. The structures of these compounds have been characterized by spectroscopic methods.

INTRODUCTION

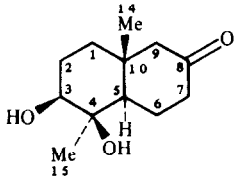
In the course of our research on the fresh whole plant of *Pluchea arguta* (syn. *Conyza odentophylla* Boiss.), we have already reported on the structures of three new sesquiterpenes [1–3] together with some known compounds. In the present communication, we wish to describe the isolation of two new sesquiterpenes (**1**, **2**) from the hexane- and ethyl acetate-soluble portions respectively of *P. arguta*. Pluchecinin (**1**) is a eudesmane having a methyl ester side chain at C-7, isolated for the first time from *Pluchea*, while **2** is a novel 11,12,13-trinor-3,4-diepicuauhtemone. Vanillic acid was also isolated from the ethyl acetate soluble portion and was identified by spectroscopic means [4, 5] as well as by direct comparison with an authentic sample.

RESULTS AND DISCUSSION

Pluchecinin (**1**) was isolated from fresh whole plant material. Its UV and IR spectra clearly indicate that the skeletal system of **1** is different to that to other reported eudesmane compounds [6–14] with respect to the absence of α,β-unsaturation or an α,β,β-trisubstituted ketone group. HRMS gave the exact mass of the molecular ion as 394.172, consistent with the formula $C_{21}H_{30}O_7$. Other prominent peaks were found at *m/z* 295, 264, 236 and 210. The peak at *m/z* 295.153 ($C_{16}H_{23}O_5$) corresponded to the loss of epoxyangelate from the molecular ion, while the peak at *m/z* 264.136 ($C_{15}H_{20}O_4$) suggested the loss of 31 mass units (OMe).



1



2

The ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) showed two downfield singlets at δ 5.52 and 5.98, each integrating for 1H, corresponding to the exomethylene group of the side chain in the molecule. Another singlet for 3H resonated at δ 3.72 due to the OMe group of the side chain. The attachment and stereochemistry of the side chain was deduced as 7β by the presence of a *dd* at δ 2.92 with a coupling constant of 3 and 9.5 Hz, assigned to H-7α. The characteristic signals for epoxy-angelate appeared at δ 3.06 (1H, *q*, $J=6$ Hz, H-3'), 1.34 (3H, *d*, $J=6$ Hz, Me-4') and 1.60 (3H, *s*, Me-5'). The position and stereochemistry of the epoxyangelate ester was deduced by the H-3 signal at δ 5.09 (*dd*, $J=6$ and 11.2 Hz) which clearly indicates the β-orientation of epoxyangelate at C-3 of the molecule. The stereochemistry at C-4 was established through the chemical shift of CH_3-15 (δ 1.25), which revealed the α-orientation of the OH function at C-4 [15]. H-5α gave a doublet of doublets at δ 2.85 having coupling constants of 2.75 and 8.5 Hz.

Two dimensional NMR measurements were carried out to verify the ^{1}H NMR assignments. The coupling interactions were established through correlated spectroscopy (COSY-45) while the multiplicities of the overlapping proton signals were determined from the 2D *J*-resolved spectrum. The assignment for the C-7 proton at δ 2.92 was thus confirmed by its COSY-45 spectrum which showed a strong cross peak with the signal at δ 3.25 (CH_2-6).

The ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) showed the presence of 21 carbon atoms in the molecule. The signal, the exomethylene group appeared at δ 124.13. The carbonyl carbon of the methyl ester resonated at δ 174.01, while OMe appeared at δ 50.80. The signals for quaternary carbons appeared at δ 144.30 (C-11) and δ 71.52 (C-4). The methine function of C-7 was assigned at δ 37.24. The

Table 1. ^{13}C NMR data for pluchecinin (**1**) ($CDCl_3$, 100 MHz)

C	ppm	C	ppm
1	32.08	12	174.01
2	23.31	13	124.13
3	79.25	14	18.21
4	71.52	15	22.42
5	46.85	1′	168.21
6	29.41	2′	60.22
7	36.24	3′	59.85
8	205.81	4′	14.59
9	58.90	5′	19.45
10	32.82	OCH_3	50.80
11	144.30		

The status of each carbon was confirmed by a DEPT experiment.

^{13}C chemical shift assignments were confirmed by hetero-COSY spectrum and are presented in Table 1. The above findings revealed that pluchecinin (**1**) is a new diester derivative of cuauhtemone having epoxyangelate esterified at C-3 and a methyl ester side chain at C-7.

11,12,13-Trinor-3,4-diepicuauhtemone (**2**) was also isolated from the fresh whole plant of *P. arguta*. Its UV spectrum showed only end-absorption at 208 nm indicating the absence of an α,β-unsaturated ketone or α,β,β-trisubstituted ketone group, in the molecule. The HRMS gave $[M]^+$ at *m/z* 212.141 corresponding to the molecular formula $C_{12}H_{20}O_3$, and suggesting a trinor-sesquiterpene character for the molecule. The ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) showed only two methyl signals instead of four. They appeared at δ 0.87 and 1.12 and correspond to Me-14 and Me-15 respectively. A ^{13}C NMR DEPT experiment also showed the signals for two methyl groups instead of four. These, findings confirmed the trinor-sesquiterpene skeleton of the molecule. A multiplet, centred at δ 2.20 was due to the methylene protons of C-9. A doublet of doublets resonated at δ 2.0 (J = 3.5, 9.3 Hz) was assigned to H-5α. H-3α appeared as a doublet of doublets at δ 3.56 (J = 3.04, 12 Hz) suggesting the β-orientation of the OH at C-3. The chemical shift of 15-Me at high field (δ 1.12) suggested the α-orientation of 15-Me at C-4 and β-orientation of the OH group [1].

The multiplicities of the proton signals were determined through 2D J-resolved spectrum, while the coupling interactions were established by COSY-45 spectrum. A strong cross peak was observed at δ 1.82 and 3.56, showing the coupling interactions between C-2 and C-3 protons. The ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) indicated the presence of two methyl signals at δ 19.01 and 21.63 which were assigned to Me-14 and Me-15 respectively. The signals at δ 59.63, 47.50 and 39.50 corresponded to C-9, C-5 and C-10 respectively on the basis of their chemical shifts and multiplicities, while the assignments of the remaining methylene groups at δ 34.53 (C-1), 26.88 (C-2) and 22.9 (C-6) were made by comparison with data reported for the same carbons in cuauhtemone [6]. There is one more signal for a methylene carbon in the DEPT spectrum at δ 42.58 which was assigned to C-7. The absence of a C-11 signal in the broad band spectrum confirmed the trinor-eudesmane character of the molecule. The ^{13}C chemical shift assignments were confirmed by hetero-COSY spectrum.

EXPERIMENTAL

Extraction and isolation of pluchecinin (**1**). Fresh plant material of *P. arguta* (8 kg) was collected from Karachi, and identified by Personnel of the Botany Department, University of Karachi (a voucher specimen has been deposited to the herbarium of the Botany Department). The plant was crushed, soaked in hexane, and homogenized with an Ultra-Turrax homogenizer. The hexane extract after removal of the solvent *in vacuo* afforded a greenish gummy mass which was chromatographed on a large silica gel column eluted successively with hexane, hexane–$CHCl_3$ mixtures, $CHCl_3$, $CHCl_3$–EtOAc mixtures, EtOAc–MeOH mixtures and finally with MeOH. Fractions eluted from $CHCl_3$–EtOAc (40–60) contained a mixture of sesquiterpenes. This mixture was subjected to repeated flash CC with $CHCl_3$–Me_2CO (3:2). The first few fractions afforded pure pluchecinin (**1**), 20 mg, as a colourless gum. The purity of **1** was confirmed on HPTLC ($CHCl_3$–MeOH 4:1) as well as on HPLC using an RP-18 cartridge with MeOH–H_2O (4:1) as mobile phase.

Pluchecinin (**1**). Colourless gum, $[\alpha]_D^{20}$ −201° (MeOH; *c* 0.01). UV λ_{max}^{MeOH} nm: 209; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1730 (epoxyangelate's carbonyl) and 1710 (ketone) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.01 (*s*, 3 × H-14), 1.25 (*s*, 3 × H-15), 1.34 (*d*, J = 6 Hz, 3 × H-4′), 1.60 (*s*, 3 × H-5′), 2.38 (*br s*, 2 × H-9), 2.92 (*dd*, J = 3 and 3 Hz, 9.5 Hz, H-7α), 2.85 (*dd*, J = 2.75 and 9.5 Hz, H-5α), 3.06 (*q*, J = 6 Hz, H-3′), 3.20 (*dd*, J = 2.5 Hz, J_{gem} = 13.4 Hz, H-6), 3.25 (*dd*, J = 3.7 Hz, J_{gem} = 13.4 Hz, H-6), 3.72 (*s*, 3H, OMe), 5.09 (*dd*, J = 6 and 11.2 Hz, H-3), 5.52 (*s*, 1H, H-13), 5.98 (*s*, 1H, H-13); FDMS: *m/z* 394; HRMS: *m/z* 394.172 ($C_{21}H_{30}O_7$, calcd. 394.170) $[M]^+$, 295.153 ($C_{16}H_{23}O_5$, calcd 295.154) $[M-Epang]^+$, 280.130 ($C_{15}H_{20}O_5$, calcd 280.131) $[M-Epang-Me]^+$, 264.136 ($C_{15}H_{20}O_4$, calcd 264.136) $[M-Epang-MeO-CO]^+$, 218.131 ($C_{14}H_{18}O_2$ calcd 218.130) $[M-Epang-MeO-CO-H_2O]^+$, 210.125 ($C_{12}H_{18}O_3$ calcd 210.125) $[M-Epang-$side chain$]^+$; ^{13}C NMR: See Table 1.

Isolation of 11,12,13-*trinor*-3,4-*diepicuauhtemone* (**2**). After extraction with hexane (twice), the residue was soaked in dist. EtOH. The ethanolic extract, after removal of the solvent *in vacuo* and extraction with Et_2O, was partitioned with EtOAc and H_2O. The EtOAc layer was separated and the aq. phase was further extracted with EtOAc. The EtOAc soluble portion was taken to dryness in a rotary evaporator. The gummy residue (20 gm) was loaded on to a large silica gel column which was developed as described above for **1**. Compound **2** was eluted with $CHCl_3$–EtOAc (3:7) and was further purified by HPLC (RP-8 column, MeOH–H_2O 7:3). Pure **2**, 12 mg, was crystallized from MeOH, mp 180°.

11,12,13-*Trinor*-3,4-*diepicuauhtemone* (**2**). Colourless crystals, mp 180° $[\alpha]_D$ +142.85, ($CHCl_3$; *c* 0.014). UV λ_{max}^{MeOH} nm: 208; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH) and 1700 (ketone) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 0.87 (*s*, 3 × H-14), 1.12 (*s*, 3 × H-15), 1.82 (*m*, 2H-2), 1.84 (*m*, H-1), 2.0 (*dd*, $J_{5'6\alpha}$ = 3.5 Hz, $J_{5'\alpha}$ = 9.3 Hz, H-5α), 2.2 (*m*, 2H-9), 2.30 (*m*, H-6), 2.4 (*m*, 2H-7), 3.56 (*dd*, $J_{3'2\beta}$ = 3.04 Hz, $J_{3'2\alpha}$ = 12 Hz, H-3α); FDMS: *m/z* 212; HRMS: *m/z* (rel. int.) 212.141 ($C_{12}H_{20}O_3$, calcd. 212.141) $[M]^+$ (3), 194.130 ($C_{12}H_{18}O_2$, calcd. 194.130) $[M-H_2O]^+$ (14), 167.107 ($C_{10}H_{15}O_2$, cacld. 167.107) $[M-C_2H_5O]^+$ (14.1), 153.091 ($C_9H_{14}O_2$, calcd. 153.099) $[M-C_3H_6O]^+$ (70), 111.081 ($C_7H_{11}O$, cacld. 111.080 $[M-C_5H_{10}O_2]^+$ (20); ^{13}C NMR ($CDCl_3$, 100 MHz): 34.53 (C-1), 26.88 (C-2), 75.16 (C-3), 73.87 (C-4), 47.50 (C-5), 22.99 (C-6), 42.58 (C-7), 214.32 (C-8), 59.63 (C-9), 39.50 (C-10), 19.01 (C-14), 21.63 (C-15).

REFERENCES

1. Ahmad, V. U. and Fizza, K. (1986) *Phytochemistry* **25**, 949.
2. Ahmad, V. U. and Fizza, K. (1987) *Liebigs. Ann. Chem.* **7**, 643.
3. Ahmad, V. U. and Fizza, K. (1988) *Phytochemistry* **27**, 1861.
4. Windholz, M. (1976) *The Merck Index*, **9**, 9590.
5. Briemair, E., Hass, G. and Voelter, W. *Atlas of Carbon-13 NMR Data*, Vol. 2, 1909.
6. Nakanishi, K., Crouch, R., Miura, I., Dominguez, X., Zamudio, A. and Villarreal, R. (1974) *J. Am. Chem. Soc.* **96**, 609.
7. Bohlmann, F. and Zdero, C. (1976) *Chem. Ber.* **109**, 2653.
8. Bohlmann, F. and Mahanto, P. K. (1978) *Phytochemistry* **17**, 1189.
9. Chiang, M. T., Bittner, M., Silva, M., Watson, W. H. and Sammes, P. G. (1979) *Phytochemistry* **18**, 2033.
10. Bohlmann, F., Ziesche, J., King, M. R. and Robinson, K. (1980) *Phytochemistry* **19**, 969.
11. Arraga-Giner, F. J., Borges-Der-Castillo, J., Manresa-Ferrero, M. T., Vazquez-Bueno, P., Rodriguez, F. and Iraheta, S. V. (1983) *Phytochemistry* **22**, 1767.
12. Sibabrata, M., Geoffery, C. A., Nisir, R., Supreeya, R., Payom, T. and Hylands, P. J. (1983) *J. Nat. Prod.* **46**, 671.
13. Arriaga, F. J. and Borges-Del-Castillo, J. (1985) *Planta Med.* **3**, 290.
14. Jakupovic, J., Misso, L. N., Chao, T. V., Thir, Bohlmann, F. and Castro, V. (1985) *Phytochemistry* **24**, 3053.
15. Chiang, M. T., Bittner, M., Silva, M., Watson, W. H. and Sammes, P. G. (1979) *Phytochemistry* **18**, 2033.