Three New Eudesmane Sesquiterpenes from Pluchea arguta

Viqar Uddin Ahmad, Tanveer Ahmed Farooqui, Kaniz Fizza, Azra Sultana, and Rasheeda Khatoon

J. Nat. Prod., **1992**, 55 (6), 730-735• DOI: 10.1021/np50084a004 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 6, pp. 730-735, June 1992

THREE NEW EUDESMANE SESQUITERPENES FROM *PLUCHEA ARGUTA*

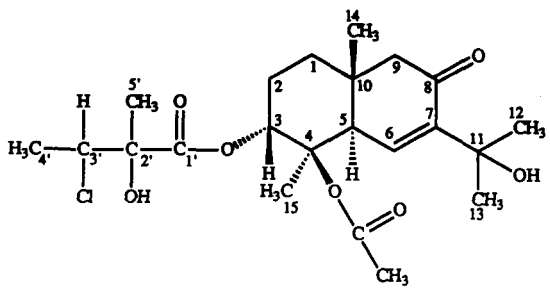
VIQAR UDDIN AHMAD,* TANVEER AHMED FAROOQUI, KANIZ FIZZA, AZRA SULTANA, and RASHEEDA KHATOON

H.E.J. Research Institute of Chemistry, University of Karachi, Karachi 75270, Pakistan

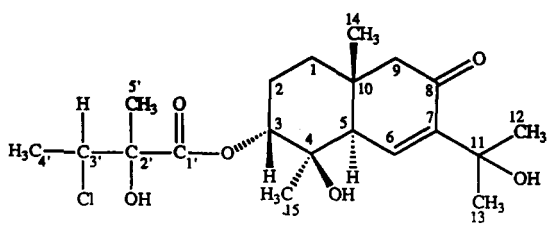
ABSTRACT.—Three new eudesmane sesquiterpenes, 3′-chloro-2′-hydroxyarguticinin [**1**], deacetoxy-3′-chloro-2′-hydroxyarguticinin [**2**], and 4,5-*epi*-cryptomeridiol [**3**], were isolated from the whole plant of *Pluchea arguta*. The structures were elucidated on the basis of spectroscopic studies.

Pluchea arguta Boiss. (syn. *Conyza odontophylla* Boiss.) (Compositae) grows as a common weed in Sind and other parts of Pakistan (1). Some *Pluchea* species are noted for their medicinal properties (2), and a number of compounds have been isolated from *Pluchea* species (3–5).

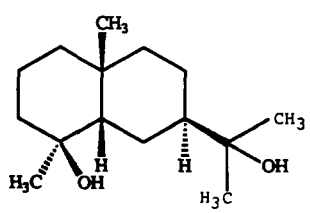
Our previous publications reported isolation and structures of several compounds from the whole plant extract (6–10). This communication deals with the isolation and structure elucidation of three new eudesmane sesquiterpenes; 3′-chloro-2′-hydroxyarguticinin [**1**], deacetoxy-3′-chloro-2′-hydroxyarguticinin [**2**], and 4,5-*epi*-cryptomeridiol [**3**]. Preliminary screening of compound **1** showed significant antibacterial activity against *Klebsiella ozaenoe*, *Proteus vulgaris*, and *Streptococcus lactis*.



1



2



3

RESULTS AND DISCUSSION

The fdms of **1** showed a molecular ion peak at *m/z* 444 (38%) with an isotopic peak at *m/z* 446 ($C_{22}H_{33}O_7{}^{37}Cl$). Hrms gave an exact mass peak at *m/z* 444.18928 attributed to molecular formula $C_{22}H_{33}O_7{}^{35}Cl$. The presence of isotopic peaks indicated the presence of a chlorine moiety in the molecule. The peak at *m/z* 427.18740 is attributed to $C_{22}H_{32}O_6Cl$, due to the loss of hydroxyl from the molecular ion; a fragment which appeared at *m/z* 408.2106 had the composition $C_{22}H_{32}O_7$ due to the loss of HCl from the molecular ion. The mass spectrum showed a major peak at *m/z* 369 $[M - HOAc - Me]^+$ (90%), supporting the presence of an ester group at C-4. This peak was indicated in a previously reported compound (11).

The ^{1}H-nmr ($CDCl_3$, 300 MHz) spectrum of **1** (Table 1) exhibited an olefinic doublet at δ 6.89 ($J = 2.0$ Hz) characteristic of H-6, suggesting the stereochemistry at C-4. It is also known (11) that when the oxygen function at C-4 has an α orientation, the H-6 signal is shifted downfield from δ 6.89 to 7.10 ppm; this is accompanied by slight upfield shifts of acetate methyl to δ 1.97 and of the Me-15 signal to δ 1.38. These chemical shifts are very similar to those reported for arguticinin (7). The signal for H-3 appeared at δ 5.92 (t, $J = 4.0$ Hz); this downfield shift indicated the attachment of an ester group at C-3. The stereochemistry at C-3 was deduced from its proton multiplicity ($J = 4.0$ Hz). Two methyl signals at δ 1.44 and 1.45 were attributed to the geminal methyls at C-11, and the Me-14 singlet appeared at δ 1.00. The secondary methyl in the ester side chain resonated at δ 1.57. The doublet due to the Me-4′ appeared at δ 1.55 ($J = 6.0$ Hz) and the H-3′ quartet appeared at δ 4.25 ($J = 6.0$ Hz). Two-di-

TABLE 1. ^{1}H-nmr Spectral Data for Compounds **1–3**.[a]

Proton	Compound		
	1	**2**	**3**
H-1	—	—	1.15 (2H, m)
H-2	1.57 (2H, m)	1.51 (2H, m)	1.48–1.52 (2H, m)
H-3	5.92 (1H, t, $J = 4.0$ Hz)	4.92 (1H, t)	1.34 (2H, m)
H-4	—	—	—
H-5	3.01 (1H, d, $J = 2.2$ Hz)	2.79 (1H, d, $J = 3.0$ Hz)	1.63 (1H, dd, $J_{5',6\alpha} = 5.2$ Hz, $J_{5,6\beta} = 11.0$ Hz)
H-6	6.89 (1H, d, $J = 2.2$ Hz)	6.92 (1H, d, $J = 3.0$ Hz)	2.01 (1H, m, H-6α) 2.05 (1H, m, H-6β)
H-7	—	—	1.65 (1H, m)
H-8	—	—	1.72 (2H, m)
H-9	—	2.43 (2H, m)	1.78 (2H, m)
H-10	—	—	—
H-11	—	—	—
H-12	1.45 (3H, s)	1.46 (3H, s)	1.26 (3H, s)
H-13	1.44 (3H, s)	1.43 (3H, s)	1.27 (3H, s)
H-14	1.00 (3H, s)	1.07 (3H, s)	0.89 (3H, s)
H-15	1.38 (3H, s)	1.18 (3H, s)	1.08 (3H, s)
H-1′	—	—	—
H-2′	—	—	—
H-3′	4.25 (1H, q, $J = 6.0$ Hz)	4.47 (1H, q, $J = 6.68$ Hz)	—
H-4′	1.55 (3H, d, $J = 6.0$ Hz)	1.57 (3H, d, $J = 6.69$ Hz)	—
H-5′	—	1.48 (3H, s)	—
(H-OAc)	1.97 (3H, s)		

[a] ^{1}H-nmr spectra were recorded in $CDCl_3$ at 300 MHz for compounds **1** and **2** and at 400 MHz for compound **3**.

mensional ^{1}H nmr was used to assign chemical shifts of the proton signals. Multiplicities of the proton signals were determined through a 2D *J*-resolved spectrum, while coupling interactions were established by a COSY-45 spectrum.

In the ^{13}C-nmr spectrum (Table 2) carbons C-2′ and C-3′ gave signals at δ 71.93 and 62.13, while in arguticinin these two carbons resonated at δ 59.90 and 59.62. It is known that when the carbon bears a Cl atom, its signal is shifted downfield due to deshielding. Assignments of these two carbons were confirmed by a DEPT experiment. Attachment of a Cl atom at C-3′ was also confirmed by these chemical shifts. Assignments for side chain, with respect to arguticinin, clearly indicated that there were some differences in the ester groups. The literature (12–15) confirmed that the ester group in **1** was 3′-chloro-2′-hydroxy-2′-methyl butyrate instead of 2′,3′-epoxy-2-methyl butyrate as in arguticinin. The ^{13}C-nmr spectrum also showed the presence of a conjugated keto group (δ 200.07) and two sp^2 carbon atoms (δ 145.40 and 141.22) which were assigned to C-7 and C-6. The oxygen-bearing carbon atoms resonating at δ 73.98, 81.59, and 76.76 were assigned to C-3, C-4, and C-11, respectively. Assignments for C-9, C-5, C-2, and C-1 were δ 57.94, 48.6, 22.85, and 32.41, respectively. The geminal methyl carbons, C-12 and C-13, resonated at δ 29.27 and 28.57, respectively. Two further methyl signals were due to C-15 (δ 18.92) and C-14 (δ 18.01).

The fdms of **2** showed molecular ion peaks at *m/z* 402 and 404 $[M+2]^+$ (32%), while hrms gave peaks at *m/z* 402.1799 and 404.1806 $[M+2]^+$ attributed to $C_{20}H_{31}O_6{}^{35}Cl$ and $C_{20}H_{31}O_6{}^{37}Cl$, respectively.

The ^{1}H-nmr spectrum of **2** exhibited a doublet at δ 6.92 ($J = 3.0$ Hz), which was

TABLE 2. ^{13}C-nmr Spectral Data for Compounds **1–3**[a] and Cryptomeridiol.

Carbon	Compound			
	1	**2**	**3**	Cryptomeridiol
C-1	32.41	41.83	41.47	41.1
C-2	22.85	21.30	20.28	20.2
C-3	73.98	70.08	43.65	43.6
C-4	81.59	80.61	72.65	72.3
C-5	48.6	49.30	48.84	54.4
C-6	141.22	142.05	20.69	21.5
C-7	145.40	146.21	41.98	50.7
C-8	200.07	200.84	21.40	22.5
C-9	57.94	57.86	41.65	44.7
C-10	39.14	39.13	34.34	34.60
C-11	76.76	70.89	74.7	73.0
C-12	29.27	29.49	29.54	27.2
C-13	28.57	29.82	29.84	27.4
C-14	18.01	18.82	18.66	18.7
C-15	18.92	22.06	21.95	22.7
C-1′	172.92	174.07		
C-2′	71.93	71.92		
C-3′	62.13	62.50		
C-4′	18.19	18.05		
C-5′	22.19	22.77		
OAc	169.19			
OAc	21.65			

[a]^{13}C-nmr was recorded in $CDCl_3$ at 100.6 MHz for Compounds **1** and **2** and at 100.64 MHz for compounds **3** and **4**.

characteristic for an olefinic proton at H-6; in **1** this signal appeared at δ 6.89 ($J = 2.0$ Hz). Literature (11) showed that upfield shifts of olefinic H-6 protons are characteristic for β-oxygen at C-4. The difference in chemical shifts of C-6 between **1** and **2** also indicated the presence of a hydroxyl group at C-4 in **2** instead of an acetate group as in **1**. A triplet at δ 4.92 is characteristic for a geminal proton H-3β, and from this downfield shift the stereochemistry of side chain ester was also concluded to have an α orientation (C-3). Singlets at δ 1.43 (3H) and 1.46 (3H), were assigned to geminal methyls at C-11. An upfield singlet at δ 1.07 (3H) was assigned to C-10 methyl. The methyl of ester side chain C-2′ showed a downfield singlet at δ 1.48 (3H), and a characteristic doublet at δ 1.57 ($J = 6.69$ Hz) was due to Me-4′. The quartet of H-3′ resonated on δ 4.47 ($J = 6.68$ Hz). A multiplet of H_2-9 was also observed at δ 2.43. These assignments suggested that compound **2** was a new deacetoxy derivative of **1**.

The ^{13}C-nmr spectrum of **2** showed the presence of carbonyl ester C-1′ at δ 174.07. The conjugated keto group resonated at δ 200.84. The two sp^2 carbon atoms at δ 142.05 and 146.21 were assigned to C-6 and C-7. The oxygen-bearing carbon atoms resonated at δ 70.08, 80.61, 70.89, and 71.92, which are due to C-3, C-4, C-11, and C-2′, respectively. All the chemical shifts of the 7-oxygen bearing carbons are similar to **1**, except for C-3 and C-4, which appeared in **1** at δ 73.98 and 81.59, respectively, indicating the change of functional group at C-4 and in the ester side chain. Assignments for C-9, C-5, C-2, and C-1 were δ 57.86, 49.30, 21.30, and 41.83 on the basis of their chemical shifts and multiplicities in known compounds. The geminal methyl carbons, C-12 and C-13, resonated at δ 29.49 and 29.82, respectively. The two methyl absorptions at highest field were due to C-15 (δ 22.06) and C-14 (δ 18.32). Complete assignments of all carbon atoms are shown in Table 2.

The eims of **3** did not show a molecular ion peak (*m/z* 240), but indicated a prominent fragment at *m/z* 204. The base peak at *m/z* 149 was attributed to loss of substituents at C-4 and C-7. In the hrms of compound **3**, a fragment ion was observed at *m/z* 204.1879 corresponding to the formula $C_{15}H_{24}$ (calcd 204.1877). A peak at *m/z* 222 in the fdms suggested loss of one H_2O molecule, whereas in the fabms a peak appeared at *m/z* 241 which confirmed the presence of two hydroxyl groups. Thus molecular formula $C_{15}H_{28}O_2$ was assigned to compound **3**.

The ^{1}H nmr revealed four methyl singlets at δ 0.89, 1.08, 1.26, and 1.27 due to Me-14, Me-15, Me-12, and Me-13 protons, respectively. The spectrum also indicated the absence of olefinic protons and a methine proton on a carbon bearing a hydroxyl group. A double doublet was observed at δ 1.63 ($J = 5.2$ and 11.0 Hz, H-5), and two multiplets were observed at δ 2.01 and 2.05, for H-6α and H-6β respectively. The results of 2D ^{1}H-nmr experiments fully agreed with the proposed structure **3**. Multiplicities of overlapping proton signals were determined from the 2D *J*-resolved spectrum, while the COSY-45 spectrum established the coupling interactions among vicinal protons. A pair of cross peaks were observed at δ 2.01 and 1.63 and at δ 2.05 and 1.63 showing the coupling interactions of H-6α and H-6β protons with those of H-5. A corresponding cross peak between H-6α and H-6β was also observed in COSY-45 spectrum. It also showed vicinal coupling interaction of H-6α and H-6β with H-7 at δ 1.65. The C-1 protons at δ 1.15 are vicinal to the C-2 protons at δ 1.48, and the latter showed vicinal coupling with C-3 protons at δ 1.34.

The stereochemistry of **3**, a cis fused decalin system, was ascertained by nOe difference measurements. Irradiation at δ 0.89 (H-14) resulted in 7.14% nOe, at δ 1.63 (H-5), and irradiation at δ 1.08 (H-15) resulted in 2.82% nOe, at δ 1.65 (H-7), which confirmed that the methyl at C-10 and proton at C-5 were β and the methyl at C-4 and the proton at C-7 were α-oriented.

The ^{13}C-nmr spectrum of **3** exhibited 15 carbon resonances. The multiplicities of

each carbon atom were determined by using DEPT experiments with polarization pulses at 45°, 90°, and 135°. These experiments revealed four methyl, six methylene, two methine, and three quaternary carbons in agreement with structure **3**. Compound **3** showed signals of quaternary carbons at δ 72.65 and 74.7, indicating that hydroxyl groups were attached to these two carbons. The relative configuration at C-4 was confirmed by comparison with 4-*epi*-plucheinol (6). The different values in the ^{13}C-nmr spectrum at C-4, C-5 (16,17) revealed that compound **3** was an isomer of cryptomeridiol (17) as shown in Table 2. Heterocosy (HETCOR) experiments were carried out to identify the relationship between carbons and their respective protons.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The uv spectra were obtained on a Shimadzu UV-240 spectrometer. Ir spectra were obtained on a JASCO A-302 spectrometer. The ^{1}H-nmr spectra were recorded on Bruker AM-300 and Bruker AM-400 nmr spectrometers in $CDCl_3$. The ^{13}C-nmr spectra were recorded at 100 MHz. The chemical shifts are expressed as ppm. Mass spectra were measured on Varian MAT-112 and MAT-312 spectrometers connected to a MAT-188 data system. All the above signals of the protons and multiplicities were determined through 2D*J*-resolved spectra, and coupling interactions were established by COSY-45 spectra.

Optical rotations were measured on a Polartronic-D polarimeter. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Analytical and preparative hplc were carried out on JASCO Model VL-614 having RP-8 column with a UVIEC-100-11. Purity of samples was confirmed by hptlc Si gel 60 F_{254} precoated glass plates (E. Merck).

PLANT MATERIAL.—Fresh plant material of *P. arguta* (20 kg) was collected in July 1989, in Karachi and identified by members of the Department of Botany, University of Karachi. A voucher specimen is deposited in the Herbarium of the Botany Department, University of Karachi.

EXTRACTION AND ISOLATION OF **1** AND **2**.—Fresh whole plant of *P. arguta* was soaked in hexane and homogenized with an ultra-turrax homogenizer. After removal of hexane extract (twice) from the homogenized material. The residue was soaked in distilled EtOH. Solvent was removed in vacuo and the residue obtained was extracted with Et_2O. The Et_2O fraction was evaporated in the rotary evaporator and subjected to Si gel cc eluting with hexane, hexane/$CHCl_3$, $CHCl_3$, $CHCl_3$/EtOAc, EtOAc, EtOAc/MeOH, and MeOH. Elution with $CHCl_3$-EtOAc (70:30) furnished **1** and **2**. Both sesquiterpenes were purified by hplc on an RP-8 analytical column using MeOH-H_2O (70:30) and MeOH-H_2O (50:50) as mobile phase for **1** and **2**, respectively.

3′-Chloro-2′-hydroxyarguticinin [**1**].—Compound **1** (17.05 mg) was isolated as a colorless solid: mp 140–145°; $[\alpha]^{29}$D +17.52 (*c* = 0.2, $CHCl_3$); ir ($CHCl_3$) 3500 (OH), 1730 (C=O, ester), 1660 cm^{-1} (α,β-unsaturated ketone); uv (MeOH) λ max 238 nm; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; fdms *m/z* 444; hrms *m/z* (rel. int. %) 444.1892 (38) ($C_{22}H_{33}O_7Cl$), $[M-OH]^+$ 427 (12) (calcd 427.1874 for $C_{22}H_{32}O_4$), $[M-HCl]^+$ 408 (7) (calcd 408.2106 for $C_{22}H_{32}O_7$), $[(M-OH-AcO]^+$ 384 (32) (calcd 384.1694 for $C_{20}H_{29}O_5Cl$), 369 (90) (calcd 369.1466 for $C_{19}H_{26}O_5Cl$).

Deacetoxy-3′-chloro-2′-hydroxyarguticinin [**2**].—Compound **2** (18.01 mg) was obtained as colorless gum: $[\alpha]^{29}$D +20 (*c* = 0.01, $CHCl_3$); ir ($CHCl_3$) 3400 (OH), 2850 (OH), 1720 (C=O, ester), 1650 cm^{-1} (α,β-unsaturated ketone); uv (MeOH) λ max 239 nm; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; fdms *m/z* 402; hrms *m/z* (rel. int. %) 402 (32) (calcd 402.1799 for $C_{20}H_{31}O_6{}^{35}Cl$), 404 (19) (calcd 404.1806, $C_{20}H_{31}O_6{}^{37}Cl$); eims *m/z* (rel. int. %) $[M-H_2O]^+$ 384 (7), $[M-Me-H_2O]^+$ 369 (45), $[M-RCOOH-Me]^+$ 235 (15), $[M-RCOOH-Me-H_2O]^+$ 217 (20), $[C_{10}H_{13}O]^+$ 149 (65).

ISOLATION OF **3**.—After extraction with Et_2O, the Et_2O-insoluble portion was partitioned between EtOAc and H_2O. The EtOAc layer was separated and the aqueous phase was further extracted with EtOAc. The EtOAc-soluble portion was evaporated, and the gummy residue was loaded on a large Si gel column. The column was developed as described above for **1** and **2**. Compound **3** was eluted using pure $CHCl_3$ and was further purified by repeated cc.

4,5-epi-*Cryptomeridiol* [**3**].—Compound **3** was obtained as white crystals, mp 104°, $[\alpha]^{29}$D +66.66 (*c* = 0.015, $CHCl_3$); uv (MeOH) λ max 273 nm; ir ($CHCl_3$) 3500 (OH), 1385 cm^{-1} (gem dimethyl); ^{1}H see Table 1; ^{13}C nmr see Table 2; positive fabms *m/z* 241; fdms *m/z* 222; hrms *m/z* (rel. int. %) 222.1985 (60) (calcd 222.1983, $C_{15}H_{26}O$); eims *m/z* (rel. int. %) $[M-2H_2O]^+$ 204 (24), $[M-2H_2O-Me]^+$ 189 (21), $[Me_2COH]^+$ 163 (10), $[M-C_4H_{11}O_2]^+$ 149 (100), $[M-C_5H_{13}O_2]^+$ 135 (30).

ACKNOWLEDGMENTS

The authors wish to thank the Pakistan Science Foundation for a financial grant (No. S-KU/CHEM/205).

LITERATURE CITED

1. R.R. Stewart, in: "Flora of West Pakistan." Ed. by E. Nasir and S.I. Ali, Fakhri Printing Press, Karachi, 1972, p. 768.
2. S. Mukhopadhyay and G.A. Cordell, *J. Nat. Prod.*, **46**, 672 (1983).
3. M.T. Chiang, M. Bittner, M. Silva, W.H. Watson, and P.G. Sammes, *Phytochemistry*, **18**, 2033 (1979).
4. F. Bohlmann and P.K. Mahanta, *Phytochemistry*, **17**, 1189 (1978).
5. K. Nakanishi, R. Gouch, J. Micera, X.A. Doringuez, A. Zamudio, and R. Villarred, *J. Am. Chem. Soc.*, **96**, 609 (1974).
6. V.U. Ahmad and K. Fizza, *Phytochemistry*, **25**, 949 (1986).
7. V.U. Ahmad and K. Fizza, *Phytochemistry*, **27**, 1861 (1988).
8. V.U. Ahmad and K. Fizza, *Liebigs Ann. Chem.*, 643 (1987).
9. V.U. Ahmad, K. Fizza, and A.R. Amber, *J. Nat. Prod.*, **52**, 861 (1989).
10. V.U. Ahmad, A. Sultana, and K. Fizza, *Z. Naturforsch.*, **45b**, 385 (1990).
11. F.J. Arriagag-Giner, J. Borges-Del-Castillo, M.T. Manresa-Ferrero, P. Vazquez-Bueno, F. Rodriguez, and S.V. Iraheta, *Phytochemistry*, **22**, 1767 (1983).
12. A. Bax and R. Freeman, *J. Magn. Reson.*, **44**, 592 (1981).
13. W.P. Aue, J. Harhan, and R.R. Ernst, *J. Chem. Phys.*, **64**, 4226 (1976).
14. G. Wagner, A. Kumar, and K. Wutrich, *Eur. J. Biochem.*, **114**, 375 (1981).
15. Atta-ur-Rahman, "Nuclear Magnetic Resonance," Springer-Verlag, New York, 1986.
16. F.E. Evans, D.W. Miller, T. Cairns, G.V. Baddeley, and E. Wenkert, *Phytochemistry*, **21**, 937 (1982).
17. M.A. Irwin and T.A. Geissman, *Phytochemistry*, **12**, 849 (1973).

Received 15 August 1991