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STRUCTURE OF A NEW GUAIANOLIDE, VESTENOLIDE, FROM *VICOA VESTITA*[1]

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ABSTRACT.—A new guaianolide, vestenolide, has been isolated from *Vicoa vestita*. Its structure has been elucidated mainly by spectroscopic methods and chemical transformations.

Recently we reported the isolation of a new guaianolide, vestolide, from *Vicoa vestita* Benth ex. Hook. f. (syn. *Inula vestita* Wall ex. DC) (Compositae) (1). The present paper deals with the structure elucidation of another guaianolide designated as vestenolide (**1**) from this plant.

RESULTS AND DISCUSSION

Vestenolide (**1**) was obtained as colorless needles, mp 194°, $[\alpha]_D$ −103.23 (C, 0.5, MeOH), $C_{15}H_{20}O_4$ (M^+ 264). Its insolubility in 10% aqueous Na_2CO_3 solution and free solubility in hot aqueous NaOH solution indicated the presence of a lactone moiety. This was corroborated by the ir spectrum of **1** which displayed absorption bands at 1725 and 1660 cm^{-1} for an α,β-unsaturated lactone. The presence of a secondary hydroxyl group and a hindered hydroxyl group was evident from the formation of monoacetate (**2**) (M^+ 306), mp 150°, ν max 3440 cm^{-1} (OH) as the major product with traces of the diacetate (**3**) (M^+ 348) on acetylation with Ac_2O/pyridine.

The molecular formula of this compound required it to be bicyclic, and since its ^{1}H-nmr spectrum (see below) did not show any angular methyl group, it was considered to be sesquiterpene lactone of guaiane series.

The ^{1}H-nmr spectral data of vestenolide (**1**) and its monoacetate (**2**), which are recorded in Table 1, showed the presence of a quaternary methyl group on a carbon bearing the tertiary hydroxyl group, a vinylidene group, a secondary hydroxyl group, and a

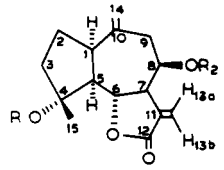
TABLE 1. ^{1}H-nmr Spectra of Compounds **1**, **2**, and **4**[a]

	1	**2**		**4**
	$\delta CDCl_3$	$\delta CDCl_3$	$\delta Eu(fod)_3$	$\delta CDCl_3$
H-5α	2.30 dd	2.32 dd	2.86 dd	—
H-6β	4.4 dd	4.4 dd	4.84 dd	3.40 d
H-7α	2.88 m	3.08 m	3.20 m	—
H-8α	4.35 m[b]	5.41 m	6.01 m	—
H-9α	2.01 dd	2.19 dd	2.31 dd	—
H-9β	2.50 dd	2.70 dd	3.09 dd	—
H-13a	5.64 d	5.47 d	5.75 d	1.5 s
H-13b	6.40 d	6.21 d	6.44 d	—
H-14	5.06 bs	4.83 bs	5.03 bs	5.16 bs
H-14′	5.15 bs	5.03 bs	5.20 bs	5.20 bs
H-15	1.34	1.34 s	1.70 s	1.24 s
-COC*H*$_3$	—	2.0 s	2.39 s	—

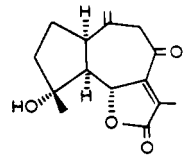
[a]*J* (Hz) for **1**,**2**: 1,5α=3.5; 5α,6β=10, 6β,7α=9, 7α,13a=3; 7α,13b=3.5; 8α,9α=3 (for **1**), 3.5 (for **2**); 8α,9β=4, 9α,9β=14. For **4**, 5,6=6.

[b]W½=8 Hz.

[1]CDRI Communication no. 3566.



1 $R_1=R_2=H$
2 $R_1=H$, $R_2=Ac$
3 $R_1=R_2=Ac$



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lactonic exocyclic methylene. Inspection of Table 1 showed that the J values of H-13a and H-13b were 3 and 3.5 Hz, respectively, which suggested the lactone ring junction to be *trans* (2). Further, vestenolide exhibited a negative cotton effect, $[\theta]_{245}=-1202$, which supported the lactone ring closure towards C-6 (3).

The relative positions and spin-spin interactions of various protons were established with the aid of decoupling and INDOR experiments with the monoacetate (**2**). When the doublet of H-13b at δ 6.21 was irradiated, the multiplet centered at δ 3.00 registered a change in the splitting. This was, therefore, confirmed to be the signal for H-7. Similarly, when the irradiating frequency was set at δ 5.45 causing simultaneous irradiation of H-13a and the carbinol proton, it simplified the multiplet of the H-7 signal much more than that observed on irradiating H-13b. This observation led to the conclusion that H-7, apart from having an allylic relationship with H-13a, was also coupled with the carbinol proton. During this irradiation, the double doublets centered at α 2.19 ($J=3.5$ and 14 Hz) and 2.70 ($J=4$ and 14 Hz) also collapsed to doublets ($J=14$ Hz each, geminal coupling). This indicated that the carbinol proton had two methylene protons in its vicinity. These revelations narrowed down the position of the secondary hydroxyl group to C-8 and lent further support to the lactone ring closure at C-6. The two double doublets at δ 2.19 and 2.70 evidently belonged to C-9 methylene protons. Irradiation of H-7 at δ 3.08 changed the H-13a and H-13b doublets to singlets and also reduced the broadening of the multiplet of the carbinol proton which further confirmed the assignments made earlier. The narrow signal width ($W½=8$ Hz) of the carbinol proton H-8 suggested that it was coupled with H-7 by a small J value. In the guaiane skeleton, H-7 is assigned an α-configuration; therefore, H-8 was also given an α-configuration so as to explain their weak spin interactions. Hence, the acetoxyl group at C-8 would acquire a β-configuration. The irradiation of H-7 also caused the double doublet at δ 4.44 ($J=9$ and 10 Hz) to change into a doublet ($J=10$ Hz) which revealed that H-6 and H-7 coupled with each other by a J value of 9 Hz. This magnitude of the J value required a *trans*-biaxial relationship between H-6 and H-7 and thus further confirmed the *trans*-junction of the lactonic ring as derived on the basis of Samek's rule. Residual coupling (10 Hz) of H-6 must be due to its interaction with H-5. In order to substantiate this conclusion, the double doublet of H-6 at δ 4.44 was also irradiated, and the region δ 2.0-3.5 was scanned to find out the signals affected. This irradiation not only narrowed the multiplet of H-7 to a large extent but also brought about the collapse of the H-5 double doublet ($J=3.5$ and 10 Hz) at δ 2.52 to a doublet ($J=3.5$ Hz). This change again showed that H-5 coupled with H-6 by 10 Hz, and its residual J value of 3.5 Hz was due to its coupling with H-1, which was the only other neighboring proton. The large J value of 10 Hz was consistent with the *trans* biaxial disposition of H-5 and H-6. Moreover, the magnitude of coupling (3.5 Hz) between H-1 and H-5 clearly suggested a *cis*-fusion of five and seven membered rings (4) in vestenolide.

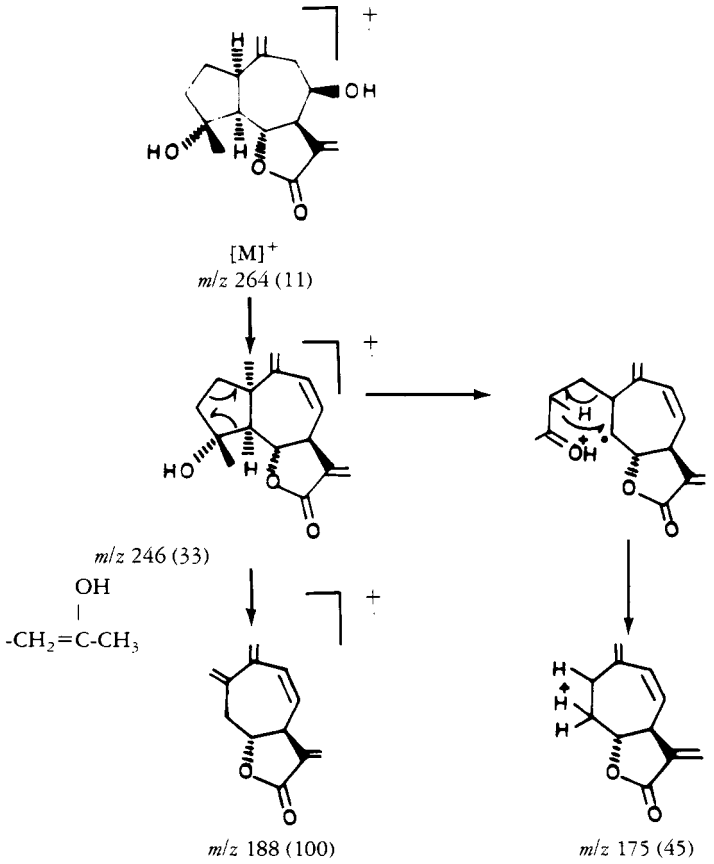
In the INDOR studies, when the monitoring field was set at δ 5.47, the position of one of the arms of H-13a signal and the carbinolic proton, it led to the appearance of

partially inverted signals at δ 3.08, 2.70, and 2.19, which were assignable to H-7, H-9β, and H-9α, respectively. Similarly, when the monitoring field was set at δ 2.72, the frequency of one of the components of the H-9β doublet doublet, a partially inverted multiplet at the carbinolic proton was observed at δ 5.41. On setting the monitoring field at the frequency of the lowest field component of the H-6 double doublet at δ 4.54, only H-5 and H-7 multiplets were observed in the spectrum. Conversely, the irradiation at one of the peaks of H-5 signal (δ 2.45) resulted in the appearance of partly inverted double doublets of H-6. Thus, the observations made in these experiments substantiated the inferences drawn from the decoupling experiments.

The position of the quaternary methyl group was established by observing the nOe on H-6. When the irradiation frequency was set at the resonance position of the quaternary methyl group at δ 1.34, the H-6 signal was found to experience about 15% enhancement in its intensity, suggesting a close proximity between H-6 and the quaternary methyl group. This necessitated the location of the quarternary methyl and the tertiary hydroxyl group at C-4 with the methyl group possessing the same configuration as H-6, i.e., β.

Finally, the unusual downfield position of the C-9 proton signals at δ 2.19 and 2.70 called for placement of the vinylidene group at C-10 (5).

The ^{1}H-nmr spectrum of the monoacetate (**2**) was also recorded after adding the lanthanide shift reagent, Eu(fod)$_3$ in order to resolve the partially overlapping signals lying in the high field region of the spectrum. Although the molecule offered more than one site of coordination for the LSR and thus had a limited utility for making signal



SCHEME 1.

assignments, it nevertheless furnished some useful information. The signal of H-6 shifted downfield by 0.40 ppm due to the presence of a β-acetoxy function at C-8, but the signal of H-5, which had a *trans*-biaxial relationship with H-6, shifted slightly more (0.54 ppm) than the latter. This furnished a positive proof of an α-hydroxyl group dangling at C-4.

Thus, the structure of vestendolide monoacetate was established as **2** and that of vestenolide as **1**, which was also in complete accord with its mass spectral fragmentation (Scheme 1).

The structure of vestenolide (**1**) was further supported by its oxidation with Jones' reagent, which furnished a keto product (**4**). It displayed uv maxima at 209 and 248 nm for an enone. In the ^{1}H nmr of the keto product, the doublets of exomethylene protons H-13a and H-13b were replaced by an olefinic methyl singlet at δ 1.5. There was no signal for H-7; consequently, H-6 was reduced to a doublet. These data showed that in the oxidation product of vestenolide, the exocyclic double band had migrated to take up an endocyclic position conjugated with the newly formed keto group as depicted in structure **4**.

This structure was in conformity with its fragmentation pattern in the mass spectrum. Besides a significant molecular ion at *m/z* 262, it showed two prominent ions at *m/z* 204 and 176. The ms also displayed $[M-H_2O]^+$, $[M-CH_3-H_2O]^+$, and $[M-H_2O-CH_3CO]^+$ peaks.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The source of material and general isolation sequence are given in Sachdev and Kulshreshtha (1). Ir, uv, and cd spectra were recorded in KBr and MeOH, respectively. The ^{1}H-nmr spectra were taken on a 90 MHz Perkin Elmer R-32 nmr spectrometer and mass spectra on a JEOL JMS-D-300 mass spectrometer. ^{1}H-nmr spectral data of **1**, **2**, and **4** are given in Table 1.

ISOLATION OF VESTENOLIDE (**1**).—Repeated column chromotography of the $CHCl_3$ fraction [previously described (1)] over silica gel using *n*-hexane-Me_2CO with increasing order of polarity resulted in the elution of vestenolide in *n*-hexane-Me_2CO (7:3) solvent system. Crystallization from *n*-hexane-Me_2CO gave colorless needles mp 194°, $[\alpha]_D$ −103.23 (c, 0.5, MeOH); cd (MeOH) $[\theta]_{244}$ −1202; uv λ max (MeOH) 210 nm (ε 10, 692); ir ν max (KBr) 3400, 2900, 1725 and 1660 cm^{-1}; ms *m/z* 264 (11%, M), 249 (8, M-CH_3), 248 (18, M-CH_3-H), 246 (33, M-H_2O), 231 (12, M-CH_3-H_2O), 228 (30, M-2H_2O), 213 (12, M-CH_3-2H_2O), 205 (10), 203 (27), 202 (12), 189 (33), 188 (100), 176 (16), 175 (45), 167 (21), 157 (35), 143 (76), 133 (42), 131 (55), and 105 (91). *Anal.* Calcd. for $C_{15}H_{20}O_4$.C, 68.1; H, 7.50. Found: C, 67.8; H, 7.46. Acetylation of vestenolide (**1**)-Vestenolide (**1**, 15 mg) in dry pyridine (0.25 ml) and Ac_2O (0.25 ml) was kept overnight at ambient temperature. After work-up, it provided a mixture of two products that were separated by column chromatography over silica gel. The less polar product (**3**) was minor and eluted out in $CHCl_3$-Me_2CO (99:1). The major and more polar product (**2**) was obtained from $CHCl_3$-Me_2CO (96:4) eluate.

VESTENOLIDE MONOACETATE (**2**).—The major product was crystallized from *n*-hexane-Me_2CO, mp 150°; ir ν max (KBr) 3400, 1780, 1745, 1660 cm^{-1}; ms *m/z*, 306 (13%, M), 288 (19, M-H_2O), 264 (1, M-CH_2=C=O), 246 (21, M-CH_3COOH), 228 (100, M-CH_3COOH-H_2O), 213 (26, M-CH_3COOH-H_2O-CH_3), 200 (27, M-CH_3COOH-H_2O-CO), 188 (92), 175 (23), 143 (53), 131 (36), 117 (35), 105 (28), 91 (52), 79 (33), and 71 (66).

VESTENOLIDE DIACETATE (**3**).—The minor product was identified by ms as a diacetate; ms *m/z* 348 (2%, M), 306 (4, M-CH_2=C=O), 291 (2, M-CH_3-CH_2=C=O), 288 (8, M-H_2O-CH_2=C=O), 270 (2, M-CH_2=C=O-2H_2O), 246 (20, M-2CH_2=C=O), and 228 (100, M-2CH_3COOH).

OXIDATION OF VESTENOLIDE (**1**).—Vestenolide (**1**, 15 mg) in Me_2CO (0.5 ml) was treated titrimetrically with Jones' reagent. Aqueous $NaHSO_3$ solution was added to decompose any excess reagent, diluted with H_2O, the Me_2CO removed at room temperature, and then extracted with EtOAc. The EtOAc layer was washed and dried over anhydrous Na_2SO_4 and concentrated. The product showed several spots on the (*n*-hexane-Me_2CO, 95:5) along with heavy streaking. It was purified by column chromatography over silica gel using *n*-hexane-Me_2CO in an increasing order of polarity. *n*-Hexane-Me_2CO (9:1) eluate gave the mono-oxo product (**4**, 2 mg) as a glassy mass; uv λ max (MeOH) 209 and 248 nm.

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