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SAGITTARIOL: A NEW DITERPENE FROM *SAGITTARIA SAGITTIFOLIA**

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Key Word Index—*Sagittaria sagittifolia*, Alismaceae, bicyclic diterpene; labda-7,14-dien-13(*S*), 17-diol.

Abstract—Sagittariol a new diterpene has been isolated from *Sagittaria sagittifolia* and characterized as labda-7,14-dien-13(*S*),17-diol.

INTRODUCTION

Sagittaria sagittifolia L. is an erect stemless aquatic winter herb, flowering in November–December. It is distributed throughout the plains of India [1] and has been used in China medicinally during childbirth and for skin diseases [2].

During a programme for screening Indian plants over a wide range of biological activities, anti-inflammatory activity was observed in a 50% EtOH extract of the air-dried, whole plant [3] collected locally during December. Fractionation of this extract into hexane, *n*-BuOH and H_2O soluble fractions, located the biological activity in the hexane soluble fraction. Column chromatography of this material on neutral alumina yielded amongst other compounds [4], a crystalline diterpene designated sagittariol. Data leading to the characterization of sagittariol as labda-7,14-dien-13(*S*),17-diol (1) is now reported.

RESULTS AND DISCUSSION

Sagittariol crystallized as white needles from C_6H_6-hexane, mp 109°, $[\alpha]_D + 41°$. It analysed for $C_{20}H_{34}O_2$ (M^+ 306) and had no UV absorption above 214 nm. Its IR and NMR spectra suggested its diterpenoid nature. Its IR spectrum had absorption bands at 3521, 3289 (OH), 1642, 1416, 940, 934 ($CH{=}CH_2$), 1381, 1377 ($>C(Me)_2$), 1660, 836 and 782 cm^{-1} ($C{=}CH$) and its NMR spectrum had signals for 4 tertiary $C\underline{H}_3$ at 9·22 τ (6 H, *s*), 8·87 τ (3 H, *s*), and at 8·72 τ (3 H, *s*). The signal at 8·72 τ could be assigned to a $C\underline{H}_3$ on carbon carrying an oxygen function. A broad 2 H singlet at 8·32 τ disappeared on D_2O shake and indicated the presence of 2 hydroxyls in the molecule. A hump assignable to $C\underline{H}_2OH$ methylene protons at 5·84 τ, suggested that one of the hydroxyls was primary. A multiplet at 4·37 τ (half band width 8 Hz) was assignable to a trisubstituted double bond [5]. Three vinylic protons resonated characteristically as an ABC system: H_A, 4·03; H_B, 5·0; H_C, 4·77 τ (J_{AB} = 10 Hz, J_{BC} = 2 Hz and J_{AC} = 17·4 Hz) and are typical of diterpenes of the labdane and pimarane series [6]. The above mentioned data in conjunction with mass spectral and analytical data, characterized sagittariol as a substituted labdane.

Sagittariol readily furnishes a monoacetate. The IR spectrum of this compound shows hydroxyl absorption at 3367 cm^{-1} indicating the presence of a tertiary or hindered hydroxyl function in sagittariol. The NMR spectrum of the monoacetate displayed an acetyl singlet at 7·82 τ. A paramagnetic shift of the carbinol methylene protons by 26 Hz indicated the primary nature of the acetylated hydroxyl. The signal of these protons also shifted 40 Hz downfield when trichloroacetylisocyanate [7] was added to sagittariol in the NMR tube. The signal of the Me on carbon carrying the oxygen function also shifted downfield from 8·72 to 8·34 τ and the 2 hydroxyl protons now resonated at $-3·3$

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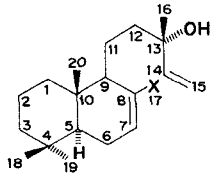
and $-3{\cdot}41\ \tau$ confirming the presence of a primary and a tertiary hydroxyl in sagittariol.

The MS of sagittariol and its derivatives were in conformity with the spectra of other bicyclic diterpenes [8]. In the spectrum of sagittariol the bicyclic fragment gives rise to a significant peak at *m/e* 207 which looses a water molecule to give the base peak of the spectrum at *m/e* 189. An abundant peak in the spectra of both sagittariol and dihydrosagittariol is at *m/e* 109. This ion is likely to be the result of a retro-Diels–Alder fission due to a 7,8 double bond and suggests the location of the ring double bond in sagittariol and the absence of substituents in ring A. The mass spectra of both sagittariol and monoacetyl sagittariol have very abundant ions at *m/e* 71. This ion, $[\mathrm{Me{-}C(OH){-}CH{=}CH_2}]^+$ arises from the side chain by fission of the C_{12}–C_{13} as the corresponding ion in the spectrum of dihydrosagittariol is at *m/e* 73. The formation of this ion is characteristic of labdane derivatives and locates the tertiary hydroxyl in sagittariol at C-13. The ion *m/e* 81 corresponding to the dehydrated side chain supports the above views.

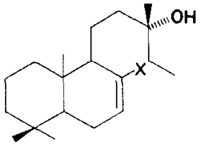
Two compounds were formed when sagittariol was refluxed with fused sodium acetate in acetic anhydride. The first, $C_{24}H_{38}O_4$ (M^+ 390), proved to be the diacetate. Its IR spectrum showed enhanced C=O and C–O stretching at 1739 and 1240 cm^{-1} respectively and no hydroxyl absorption. Its NMR spectrum had two acetyl $C\underline{H}_3$ singlets at $7{\cdot}95$ and $8{\cdot}0\ \tau$. Compared to the spectrum of the monoacetate, the only other significant difference was a paramagnetic shift of the $C\underline{H}_3$–C–O– signal from $8{\cdot}72$ to $8{\cdot}49\ \tau$.

The second compound was expectedly dehydrosagittariol acetate. It had λ_{max} 226 nm and was, therefore, likely to be the 12,14 diene rather than the 13(16),14 diene. Dehydration of sagittariol with thionyl chloride and pyridine also gave a product with λ_{max} 226 nm and established that the tertiary hydroxyl was allylic. Ozonolysis of sagittariol resulted in cleavage of the terminal side chain double bond. Liberated formaldehyde was identified through its dimedone derivative.

Hydrogenation of sagittariol in methanol in the presence of Adam's catalyst yielded dihydrosagittariol (**2**), $C_{20}H_{36}O_2$ (M^+ 308). It was clear from the NMR spectrum of this compound that the side chain double bond had been reduced as the 12 line signals for an ABC system in the olefinic region were not present. On the other hand the $\underline{H}$ of the ring double bond, which was uneffected resonated around $4{\cdot}38\ \tau$. The location of this double bond at C-7, suggested by the mass spectra of sagittariol and dihydrosagittariol, was confirmed when MnO_2 oxidation was found to given an α,β-unsaturated aldehyde $C_{20}H_{32}O_2$ (M^+ 304). This compound had λ_{max} 230 nm (ϵ, 8900), ν_{max} 3311 (OH), 1684 (–C=0), 1630 (C=CH) and 920 ($CH{=}CH_2$) cm^{-1}. Its NMR spectrum had 4 C-$C\underline{H}_3$ singlets at $9{\cdot}3$, $9{\cdot}2$, $8{\cdot}87$ and $8{\cdot}7\ \tau$, a conjugated olefine $\underline{H}$ signal at $3{\cdot}3\ \tau$ (1 H, *t*) and an aldehyde $\underline{H}$ signal at $0{\cdot}59\ \tau$. The C-9 $\underline{H}$ gave rise to a multiplet at $6{\cdot}87\ \tau$ and the C-6 $C\underline{H}_2$ to a quartet at $7{\cdot}55\ \tau$. The structure (**3**) can, therefore, be assigned to this aldehyde and sagittariol is labda-7,14-dien-13,17-diol (**1**). An alternate structure with an exocyclic double bond at C-9 is untenable as dehydrosagittariol would have been a conjugated triene.



(**1**) X = CH_2OH
(**3**) X = CHO



(**2**) X = CH_2OH
(**4**) X = Me

During studies on the hydrogenation of sagittariol, it was found that hydrogenation in the presence of Pd/C under pressure of sagittariol or dihydrosagittariol yielded a product, $C_{20}H_{36}O$ (M^+ 292). The NMR spectrum of this compound differed from that of dihydrosagittariol in having a signal for an additional C–$C\underline{H}_3$ on a double bond. The $C\underline{H}_2OH$ signal in the spectra of both sagittariol and dihydrosagittariol at $5{\cdot}84\ \tau$ was also absent. This compound is, therefore, (**4**). The reduction of such allylic primary alcohols has been reported before [9].

There is good correlation between the optical rotation and structures of labdane diterpenoids [10]. The sample of sagittariol on which the present studies were carried out appeared to be pure on the basis of TLC on $AgNO_3$ impregnated plates, spectra and m.p. but had unexpectedly high dextrorotation for the assigned structure. GLC of this material indicated 3 constituents. The major constituent, isolated by preparative GLC, had $[\alpha]_D$

+49° and after recrystallization, +41·6°. Another cycle of preparative GLC yielded material with the same optical rotation. However, as this GLC indicated the same three constituents as before, it was apparent that sagittariol was decomposing on the GLC column. This fact was confirmed by the TLC of the rechromatographed material. The most likely impurity is the Δ [8, 9] isomer, which should be strongly dextrorotatory. The NMR spectra of sagittariol and dihydrosagittariol, however, integrate for and clearly display the ring olefinic proton signal. Because of doubts about the purity of the compound, stereochemical assignments made are tentative.

The decrease in dextrorotation on reduction of the sagittariol side chain double bond suggests that the 16–Me is *β* and that the stereochemistry at 13 is *S*. This conclusion is supported by a consideration of the chemical shift of the 16-$C\underline{H}_3$, which in the case of labda-7,14-dien-13-ols have been reported at 8·76 and 8·92 *τ* in *S* and *R* compounds respectively [11]. In the spectrum of sagittariol, the signal for these protons is at 8·72 *τ*. As it is unlikely that the 17-CH_2OH would effect the chemical shift of the 16-Me markedly, sagittariol would appear to be the 13-*S* isomer and has the structure **1**.

EXPERIMENTAL

IR, UV and 60 Mcs NMR were recorded in KBr, EtOH and $CDCl_3$ with TMS as internal standard respectively unless indicated. Uncorrected capillary mp and photoelectrically measured $[\alpha]_D$ in $CHCl_3$ are reported. TLC was done on Si gel plates and on $AgNO_3$ impregnated Si gel plates when required.

Isolation of sagittariol (**1**). Air-dried, powdered, whole plant (8 kg) was extracted with 50% EtOH (20 l.) by percolation at room temp. and extract concd under red. press. at a temp. below 50°. The residue obtained was successively extracted with C_6H_{14} (10 l.), C_6H_6 (8 l.) and EtOAc (5 l.). The residue after removal of C_6H_{14} was chromatographed over neutral alumina (1·5 kg). Elution with C_6H_{14} and C_6H_{14}–C_6H_6 yielded hentriacontanone and *β*-sitosterol, while subsequent elution with C_6H_6 gave sagittariol which crystallized from C_6H_6–C_6H_{14} as needles (1·0 g), M^+ 306; mp 109–10°; $[\alpha]_D$ +41·6°. (Found: C, 78·30; H, 11·00. $C_{20}H_{34}O_2$ requires: C, 78·40; H, 11·10%). Processing of the C_6H_6 extract yielded another 0·4 g of sagittariol.

Sagittariol monoacetate. Acetylation with C_5H_5N–Ac_2O overnight and purification by chromatography over neutral alumina and then by preparative TLC gave the nonoacetate which did not crystallize. M^+ 348; ν_{max}(neat) 3367, 2865, 1739, 1656, 1468, 1381 and 1225 cm^{-1}; NMR: *τ* 9·25 (*s* 3 H), 8·8 (*s* 3 H), 8·77 (*s* 3 H), 8·7 (*s* 3 H), 8·6–8·74 (10 H), 8·4 (1 H disappears on D_2O shake), 7·94 (4 H), 7·82 (*s* 3 H), 5·4 (*s* 2 H), 4·34 (*m* 1 H), 4·98, 4·76 and 4·01 (3 H ABC – $J_{AB} = 2$, $J_{BC} = 10$, $J_{AC} =$ 17·4 Hz). (Found: C, 75·51; H, 10·1. $C_{22}H_{36}O_3$ requires: C, 75·86; H, 10·34%).

Sagittariol diacetate. Was formed by refluxing in Ac_2O–NaOAc for 4 hr under N_2. The 2 main constituents were purified over alumina and by preparative TLC but did not crystallize. The diacetate had M^+ 390; $[\alpha]_D$ +3·5°. NMR: *τ* 9·25 (*s* 3 H), 8·9 (*s* 3 H), 8·74 (*s* 3 H), 8·49 (*s* 3 H), 8·6–8·74 (*m* 10 H), 8·0 (*s* 3 H), 7·96 (4 H), 7·95 (*s* 3 H), 5·37 (*s* 2 H), 4·34, 4·98, 4·76 and 4·01 (1 H *m* and 3 H ABC 12 lines). (Found: C, 73·6; H, 9·83. $C_{24}H_{38}O_4$ requires: C, 73·83; H, 9·74%). The second product was a diene, λ_{max} 226 nm, ϵ, 15670; ν_{max}(neat) 2890, 1736, 1684, 1468, 1379, 1255, 1119, 1074, 1034, 961 and 923 cm^{-1}.

Ozonolysis of sagittariol. O_3 was passed through soln of sagittariol (50 mg) in EtOAc (20 ml) at −10° for 6 hr. The reaction soln was then allowed to warm up to room temp, stirred with Zn dust (200 mg) for 1 hr, filtered and steam distilled. HCHO was identified in the distillate by formation of a dimedone derivative mp and mixed mp 184°.

Dihydrosagittariol (**2**). Sagittariol was hydrogenated in EtOH with PtO_2 for 5 hr. The product crystallized from C_6H_{14}, mp 76–7°; M^+ 308; $[\alpha]_D$ +35·7°; ν_{max} 3311, 2907, 1468, 1441, 1381, 1344, 1136, 1122, 1104, 1037, 1001, 958, 895 and 838 cm^{-1}. NMR: *τ* 9·17 (*s* 6 H), 9·1 (*t* 3 H, *J* = 7 Hz), 8·84 (*s* 3 H), 8·8 (*s* 3 H), 8·4–8·73 (12 H), 7·89 (4 H), 5·8 (*s* 2 H), and 4·38 (*m* 1 H, half band width 8 Hz). (Found: C, 77·5; H, 11·20. $C_{20}H_{36}O_2$ requires: C, 77·9; H, 11·68%).

Labda-7 *en*-13 *ol* (17-*deoxy-dihydrosagittariol* (**4**). Dihydrosagittariol was hydrogenated in EtOH with 10% Pd/C for 22 hr. The product was purified by preparative TLC. M^+ 292, $[\alpha]_D$ + 29·2°. IR, ν_{max} (neat) 3289, 2865, 1445, 1368, 1147, 1094, 1037, 934, 895, 829, 783 and 760 cm^{-1}. NMR: *τ* 9·2 (*s* 6 H), 9·10 (*t* 3 H), 9·0 (*s* 3 H), 8·88 (*s* 3 H), 8·4–8·68 (12 H), 8·34 (*d*, 3 H, *J* = 2 Hz), 8·0 (4 H), 4·7 (*m* 1 H, half band width 8 Hz). (Found: C, 82·45; H, 12·02. $C_{20}H_{36}O$ requires: C, 82·1; H, 12·33%).

MnO_2 *oxidation of sagittariol*. Sagittariol was oxidised in C_6H_6 with active MnO_2 at room temp for 50 hr. The product was purified by chromatography over neutral alumina and by preparative TLC, but did not crystallize: $[\alpha]_D$ +41·1°.

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