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THE LOCATION OF THE METHYLOL GROUPS IN SAPOGENOL C AND ERYTHRODIOL AND ITS BIOSYNTHETIC SIGNIFICANCE

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Key Word Index—Sapogenol C; erythrodiol; stereoisomerism; ^{13}C NMR; triterpenoid biosynthesis.

Abstract—The ^{13}C NMR spectra of erythrodiol and sapogenol C have been determined and assignments made for all 30 carbons in each compound. This has permitted a hydroxyl group of sapogenol C to be located at C-24. The biosynthetic significance of sapogenol C and erythrodiol production by oxidation of β-amyrin is discussed.

Recently, we reported the biosynthesis of medicagenic acid (**1**) and sapogenols from [^{14}C]squalene and [2-^{14}C]mevalonate in soybean (*Glycine max*) and alfalfa (lucerne, *Medicago sativa*) seedlings [1]. Since these triterpenoids possess the oleanane skeleton [2], it is reasonable to assume that they are derived by stepwise oxidation of β-amyrin (**2**). Theoretically, the biosynthetic transformations of β-amyrin to medicagenic acid may proceed by hydroxylation at C-28 to erythrodiol (**3**) and further oxidation at C-28 to oleanolic acid (**4**). Additional oxidation would then occur at C-2 and C-23 to produce medicagenic acid. The presence of oleanolic acid [3] and other more highly oxidized β-amyrin derivatives [4], e.g. hederagenin (**5**) in lucerne roots supports this hypothesis. Since the incorporation of radioactivity into medicagenic acid was significantly greater than into the sapogenols in alfalfa [1, 5] and because rigorous evidence for assigning the methylol group in sapogenol C (**6**) was lacking, the possibility had previously been considered that the sapogenols are products of medicagenic acid metabolism [5]. This hypothesis is untenable in light of the results of our present communication.

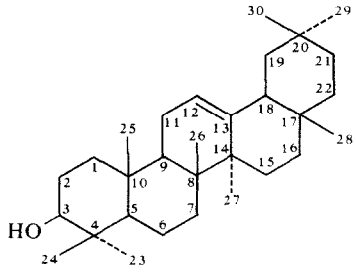
We have previously published ^{1}H NMR data on sapogenols [6], but in order to determine the position of the hydroxyl groups in sapogenol C (**6**) and erythrodiol (**3**) we have now taken advantage of ^{13}C NMR spectroscopy. Using the published ^{13}C NMR spectra of β-amyrin [7] and 23-hydroxyerythrodiol [8], we have been able to identify and assign the peaks characterizing the 30 carbon atoms in sapogenol C and erythrodiol (Table 1). Evidence for the location of the hydroxyl group of sapogenol C at C-24 rather than C-23 is derived from a comparison of the signals arising from C-23 and C-24 in sapogenol C and in 23-hydroxyerythrodiol [8].

Thus, in 23-hydroxyerythrodiol the C-24 methyl is at δ 11.6. This peak is absent in the spectrum of sapogenol C. The high field methyls are assigned to C-25 and C-26, while the methyls at δ 26.1, 31.4 and 22.4 are assigned, respectively, to C-27, C-29, and C-30 by comparison with 23-hydroxyerythrodiol. That leaves the methyl in sapogenol C at δ 24.6 assigned to C-23. The 3.9 ppm upfield shift on hydroxylation of the geminal methyl on C-4 is similar to the 3.9 ppm upfield shift for hydroxylation of β-amyrin to 23-hydroxyerythrodiol. The β-configuration for the C-3 group is assigned on the basis of known biosynthetic considerations for the cyclization of 2,3-oxidosqualene to β-amyrin [9]. The signals due to the remaining carbon atoms of sapogenol C and erythrodiol

Table 1. ^{13}C NMR chemical shifts (±0.1 ppm) for pentacyclic triterpenoids in $CDCl_3$ (ppm from TMS)

Carbon No.	Sapogenol C (**6**)	Erythrodiol (**3**)
1	38.4	38.6
2	27.7	27.2
3	80.8	79.0
4	42.3	38.8*
5	55.9	55.2
6	18.5	18.4
7	33.3	32.6
8	39.5	39.8*
9	47.8	47.6
10	36.7	36.9
11	23.8	23.6
12	122.4	122.3
13	144.4	144.2
14	42.8	41.7
15	25.6	25.6
16	29.9	22.0
17	35.0†	36.9
18	46.0	42.3
19	46.4	46.5
20	33.2†	31.0
21	134.3*	34.1
22	136.1*	31.0
23	24.6	28.1
24	64.5	15.5
25	16.1	15.5
26	16.9	16.7
27	26.1	25.9
28	28.0	69.7
29	31.4	33.2
30	22.4	23.6

*,†Assignments could be reversed.



Designation	Name	Structural features				
		C-2	C-21(22)	C-23	C-24	C-28
1	Medicagenic acid	OH	H	COOH	Me	COOH
2	β-Amyrin	H	H	Me	Me	Me
3	Erythrodiol	H	H	Me	Me	CH_2OH
4	Oleanolic acid	H	H	Me	Me	COOH
5	Hederagenin	OH	H	OH	Me	COOH
6	Sapogenol C	H	Δ	Me	CH_2OH	Me

are assigned by comparison with those reported for β-amyrin [7] and 23-hydroxyerythrodiol [8].

The sequence of biosynthetic events following the oxidation of β-amyrin has not yet been completely established, but from the occurrence of various oxidized oleanane triterpenoids in alfalfa and soybean, and from our ^{13}CNMR data for sapogenol C, the following hypothesis is proposed. Differential oxidation of β-amyrin constitutes a bifurcation in the amyrane pathways, one of which, in alfalfa, proceeds through erythrodiol and oleanolic acid to medicagenic acid. The other pathway, operating both in soybean and alfalfa, proceeds through sapogenol C to the more highly oxidized sapogenols [1, 6]. This hypothesis is supported by the difference in the location of the oxidative attack on the geminal methyl groups of β-amyrin in sapogenol C (**6**) and medicagenic acid (**1**). In the former, it occurred at the axial (4β-methyl) group, whereas in the latter a sequential oxidation of the equatorial (4α-methyl) group has taken place.

EXPERIMENTAL

Sapogenol C was isolated from *Glycine max* [6] and erythrodiol was obtained from Lab. Sarsyntex (Merignac, France). Both compounds were purified (>99%) by reversed-phase HPLC [10]. Sapogenol C emerges from the column in 10.5 min and erythrodiol in 3.8 min. EI–MS were obtained on a 70/70 F double-focusing instrument (MicroMass, VG-Organic Ltd, Altrincham, U.K.) by use of the direct probe and an ion-source temperature of 160°. The MS for sapogenol C showed *m/z* 440 $[M]^+$ (6), confirming its MW, and a diagnostic base peak at *m/z* 216 (M^+ (100), confirming the amyrane skeleton [11]. Peaks at *m/z* 224 (M^+ (7.5), 206 $[M - H_2O]^+$ (8), and 175 $[M - H_2O - CH_2OH]^+$ (8) characterize the hydroxylated substituent at C-4 in the sapogenols [6]. The MS for erythrodiol showed *m/z* 442 $[M]^+$ (3) and a base peak at *m/z* 216 (100).

The ^{13}CNMR spectra of sapogenol C and erythrodiol, both decoupled and with full proton coupling, were obtained in $CDCl_3$ on a JEOL PFT-100 (25.03 MHz) spectrometer at room temp. All chemical shifts are given in ppm downfield from internal tetramethylsilane (TMS). Other measurement conditions were as follows: 6.25 kHz spectral width, 450 pulse width, 2 sec repetition time, 16 K data points.

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