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MASS SPECTRA OF STEROIDS HAVING A LACTONE-SIDE CHAIN

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SUMMARY

The mass spectra of different steroidal compounds having a lactone-side chain were recorded. The fragmentation pattern of the tetracyclic steroid system is not modified by the structure of the lactone ring. Except for parent ion value and the losses of the substituents at C-3 and C-20, the method is apparently not useful for characterization of the lactone ring itself. The attachment position of the steroid nucleus to the lactone ring can be differentiated by the relative intensity of the peak at *m/e* 85.

INTRODUCTION

The main group of steroids possessing a lactone ring at C-17 includes the known cardiac genines cardenolides with a butenolide ring and the bufadienolides, *i.e.* C-24 homologs of cardenolides having a 20,22-unsaturated lactone ring (bufadienolide ring).

Mass spectral studies on these compounds comprise cardenolide aglycones [1], cardenolide glycosides [2] and bufadienolides [3].

A less known group of steroidal compounds that also possess lactone rings attached to C-17 includes the isocardenolides [4] which, according to most available evidence, are not naturally occurring products, although some of them have been isolated from natural sources [5]. In this example however, the possibility of artifact formation cannot be ruled out.

To our knowledge no systematic investigation has been published on compounds such as cardenolides, isocardenolides, their hydrogenation products (cardanolides and isocardanolides), nor on C-20 hydroxylated cardanolides.

In the present work we report mass spectra of several representative compounds of these types which were done on products that were synthetized in our laboratory [6, 7].

MATERIAL AND METHODS

The compounds under study are products of synthesis [6, 7] including 3β-Hydroxy-5ene-isocardanolide (**I**), 3β-Acetoxy-5ene-isocardenolide (**II**), 3β-Hydroxy-5ene-cardenolide (**III**), 3β,20-Diacetoxy-5ene-cardanolide (**IV**), 3β-Acetoxy-20-Hydroxy-5ene-cardanolide (**V**), 3β,20-Dihydroxy-5ene-cardanolide (**VI**), 3β-Acetoxy-5ene-cardanolide (**VII**) and 3β-Acetoxy-5αH-cardanolide (**VIII**).

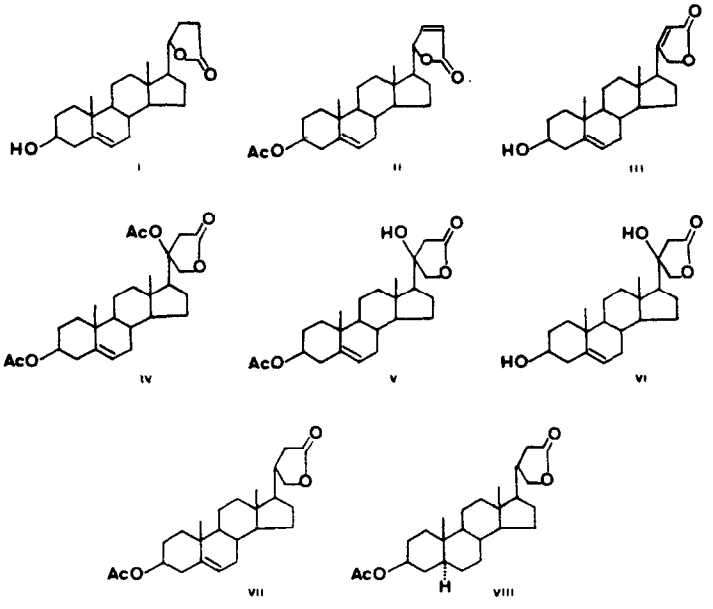

Table 1. Partial mass spectra of compounds **I–VIII**. Values of *m/e* and relative intensities as % of base peak

Ion*	I	II	III	IV	V	VI	VII	VIII
M^+	358 (88.5)	—	356 (24.3)	—	—	374 (36.7)	400 (3.4)	402 (2.0)
M-15	343 (23.8)	—	341 (7.0)	—	—	359 (8.0)	—	—
M-18	340 (87.9)	—	338 (48.4)	—	—	356 (70.8)	—	—
M-33	325 (70.6)	—	323 (52.9)	—	—	341 (33.3)	—	—
M-36	—	—	—	—	—	338 (12.3)	—	—
M-51	—	—	—	—	—	323 (15.3)	—	—
M-58	—	340 (100.0)	—	—	—	—	342 (64.0)	—
M-60	298 (15.2)	338 (43.1)	—	398 (86.4)	356 (100.0)	314 (6.4)	340 (100.0)	342 (100.0)
M-75	283 (6.0)	323 (11.4)	—	383 (2.7)	341 (11.3)	299 (7.5)	325 (22.8)	—
M-78	—	—	—	—	338 (21.7)	—	—	—
M-85	273 (75.4)	313 (0.3)	271 (20.3)	—	331 (2.2)	289 (34.4)	315 (1.2)	—
M-93	—	—	—	—	323 (10.3)	—	—	—
M-111	247 (74.1)	—	245 (27.2)	—	305 (0.5)	263 (17.0)	289 (3.3)	—
M-120	—	—	—	338 (100.0)	—	—	—	—
M-135	—	—	—	323 (51.1)	—	—	—	—
288	—	—	—	—	—	—	—	(7.2)
273	—	—	—	—	—	—	—	(5.6)
255	(10.6)	(6.0)	(43.7)	(6.0)	(10.9)	(19.4)	(15.2)	—
228	(5.0)	(2.6)	(14.4)	(21.8)	(5.5)	(5.7)	(9.2)	—
227	(9.6)	(4.7)	(9.5)	(5.5)	(2.4)	(4.9)	(7.2)	—
214	(4.6)	(3.6)	(12.6)	(8.4)	(3.2)	(7.5)	(10.1)	—
213	(18.2)	(8.7)	(61.6)	(43.8)	(13.3)	(33.1)	(36.2)	—
199	(10.9)	(5.4)	(23.4)	(8.1)	(4.0)	—	(12.5)	—
145	(65.4)	(38.1)	(75.5)	(44.3)	(30.8)	(55.4)	(60.2)	—
119	(50.9)	(26.4)	(16.6)	(6.9)	(12.9)	(40.2)	(38.9)	—
105	(77.7)	(47.4)	(85.8)	(71.2)	(35.0)	(69.1)	(74.6)	—
93	(52.7)	(36.4)	(71.8)	(54.1)	(23.4)	(63.2)	(65.6)	—
85	(100.0)	(55.8)	(7.8)	(3.1)	(2.1)	(4.6)	(14.6)	—

* 33 = 18 + 15; 36 = 18 + 18; 58 = 43 + 15; 75 = 60 + 15; 78 = 60 + 18; 85 = $C_4H_5O_2$; 93 = 60 + 18 + 15; 120 = 60 + 60; 135 = 120 + 15; 288 and 273 (see Results).

Mass spectra were determined at 70 eV by the direct insertion method with a Varian-Mat CH-7A mass spectrometer coupled to a Varian Mat Data System 166, which was programmed for automatic background substraction.

RESULTS

Table 1 presents the spectra of compounds **I–VIII** and indicates the most probable assignments to the respective ions.

The parent ion M^+ is apparently more stable with a free than with an acetylated hydroxyl group at C-3 **(I, III, VI)**. M^+ of the acetylated compounds is either very weak **(VII, VIII)** or does not appear at all **(II, IV, V)**. Apparently there is no correlation between the intensity of M^+ and the type of lactone ring.

The fragments M-15, M-18, M-33, M-36, M-51, M-60, M-75, M-78, M-120 and M-135 appear as expected in those compounds having substituents such as hydroxy and/or acetoxy at C-3 and/or at C-20, besides the loss of a methyl group [8].

The M-85 ion is found as a consequence of the loss of a saturated lactone ring. It is absent in some compounds and of variable intensity in others. Its diagnostic value is therefore questionable. All those compounds having the M-85 ion also show an M-111 ion which could be formed by loss of the saturated lactone ring plus C-16 and C-17. On the other hand, the ion M-111 has been occasionally observed in steroidal compounds having a double bond at C-5, C-6. The mechanism in this case had not been elucidated, although it is presumed that the ion is formed by rupture of ring B bonds (mainly 9,10 and 5,6); if this occurs a previous migration of the double bond must take place [9].

The ion 255 represents the tetracyclic steroidal system with two unsaturations: the 5,6 double bond and a bond formed as the result of the 1,2 loss of water or acetic acid from the group attached to C-3.

In compound **VIII**, having a saturated ring B, the most important fragmentation comes from a *retro* Diels–Alder reaction on the M-60 ion producing the fragment of *m/e* 288 which in turn loses a methyl group to give ion 273.

The ion of *m/e* 228 is formed by partial breakdown of ring D; homolysis of the C-15, C-16 bond produces a neutral olefin and ion 228. Expulsion of a methyl group (Me-19) yields ion 213 [8]. Ion 227 is partially formed from 228 by transfer of one hydrogen, mainly from C-14.

One of the most characteristic fragmentation of steroids having a side-chain at C-17 is the loss of the side-chain plus 42 units yielding the ion 213. Normally ion 214 is formed by a similar mechanism [8, 10]. The cleavage of the C-13,C-17 bond diminishes the tension of the *trans*-hidrindane system. The subsequent breaking of the C-14,C-15 bond with the transference of one hydrogen (from Me-18) produces ion 213 as an allylically stabilized carbonium

ion. The transference of two hydrogens yields ion 214. A small percent of 213 could be produced from ion 228 by loss of 15 mass units.

The fragment 199 is formed by expulsion of a methyl group (Me-19) from the ion 214 with the homolytic cleavage of C-8,C-9 bond; this fragment is stabilized as a carbonium ion with allylic conjugation [8].

Ion 145 is apparently formed by a complex mechanism with multiple transference of hydrogen [8]. It is produced by the rupture of C-8,C-14 and C-9,C-11 bonds leaving rings A and B without modifications, the loss of water or acetic acid depending on the substituent at C-3.

Ion 119 is the result of the cleavage of C-9,C-10 and C-7,C-8 bonds [11] with the charged fragment containing ring A, a portion of ring B plus Me-19.

Ions 105 and 93 can be tentatively considered as being formed by similar mechanisms yielding ions 109 and 95 from cholestane [11]. For our compounds the formation of 93 should involve a migration of C-5,C-6 double bond prior to fragmentation.

Ion 85 arises because of cleavage of substituent α to the lactonic oxygen being therefore the base peak of compound **I** and important in **II** and **VII** [12]. Its intensity allows the differentiation between cardanolides and isocardanolides and also between cardenolides and isocardenolides.

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