[Chem. Pharm. Bull. 19(5) 954—958 (1971)]

UDC 547.92.04 : 547.462.3.07

Synthesis of N-(Steroid-17-yl)-maleimide[1]

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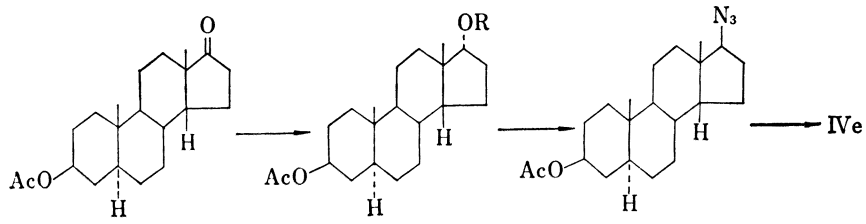
(Received October 14, 1970)

As the modified cardenolide N-(steroid-17-yl)-maleimide (VII), which is capable of reacting with SH group, has been prepared. Condensation of 17-aminosteroid (IV) with maleic anhydride gave N-(steroid-17-yl)-maleamic acid (V), which on treatment with acetic anhydride was led to VII by intramolecular dehydration (see Chart 2). Formation of isomaleimide (VI), an intermediate leading to VII, is also described.

As a part of our program dealing with the studies on the cardiotonic steroid analogs we have synthesized isobufadienolide and androstan-17-yl-isoxazole and -pyrazole.[3] It has recently been reported that 3β,5β,14β,19,21-pentahydroxypregnan-20-one 3,19-diacetate 21-iodoacetate exhibits the cardioactivity despite of lacking an unsaturated lactone ring at C-17.[4] Kupchan and his co-workers also reported that hellebrigenin 3-haloacetates are highly potent irreversible inhibitors of the (Na+K)-activated adenosine triphosphatase.[5] In addition the remarkable anti-cancer activity of some cardiotonic steroids has recently received the considerable attentions.[6] These interesting findings prompted us to examine the physiological activity of the steroidal alkylators, which are capable of reacting with SH group. The present paper describes the preparation of the titled compounds as the modified cardenolide, which possess the maleimide function, a typical SH-blocking group, at C-17 on the steroid nucleus.

The aminosteroids used as a starting material were shown in Chart 2. Of these compounds 17β-amino-5α,14β-androstan-3β-ol (IVe) has not yet been described in the literatures. Accordingly the synthesis of this compound was first carried out employing 3β-acetoxy-5α,14β-androstan-17-one (I) as a starting compound.[7] Reduction of I with sodium borohydride[7] under mild conditions gave the 3β,17α-diol 3-monoacetate (IIa), which in turn was led to the 17-tosylate (IIb) in the usual way. Reaction with sodium azide in hexamethylphosphoric triamide[8] proceeded readily to furnish the 17β-azido derivative (III) accompanying Walden inversion. Subsequent treatment with lithium aluminum hydride resulted in formation of the desired compound, IVe, which fulfills the stereochemical requirement at C-14 and C-17 for the cardiotonic activity.[3]

1) This paper constitutes part IX of the series entitled "Studies on Cardiotonic Steroid Analogs"; Part VIII: T. Nambara, K. Shimada, J. Goto, and S. Goya, *Chem. Pharm. Bull.* (Tokyo), **19**, 21 (1971).
2) Location: *Aobayama, Sendai.*
3) T. Nambara, K. Shimada, S. Goya, and J. Goto, *Chem. Pharm. Bull.* (Tokyo), **16**, 2236 (1968); T. Nambara, K. Shimada, and S. Goya, *ibid.*, **18**, 453 (1970); S. Goya, K. Shimada, J. Goto, S. Usuda, and T. Nambara, *Yakugaku Zasshi*, **90**, 537 (1970); T. Nambara, K. Shimada, T. Nemoto, and S. Goya, *Chem. Pharm. Bull.* (Tokyo), **18**, 1658 (1970); T. Nambara and K. Shimada, *ibid.*, **19**, 16 (1971).
4) M.E. Wolff, W. Ho, and H.-H. Chang, *J. Pharm. Sci.*, **57**, 1450 (1968); M.E. Wolff, H.-H. Chang, and W. Ho, *J. Med. Chem.*, **13**, 657 (1970).
5) A.E. Ruoho, L.E. Hokin, R.J. Hemingway, and S.M. Kupchan, *Science*, **159**, 1354 (1968).
6) J.E. Pike, J.E. Grady, J.S. Evans, and C.G. Smith, *J. Med. Chem.*, **7**, 348 (1964); S.M. Kupchan, R.J. Hemingway, and R.W. Doskotch, *ibid.*, **7**, 803 (1964); S.M. Kupchan, J.R. Knox, J.E. Kelsey, and J.A.S. Renauld, *Science*, **146**, 1685 (1964); R.B. Kelly, E.G. Daniels, and L.B. Spaulding, *J. Med. Chem.*, **8**, 547 (1965); S.M. Kupchan, M. Mokotoff, R.S. Sandhu, and L.E. Hokin, *ibid.*, **10**, 1025 (1967).
7) A.F.St. André, H.B. MacPhillamy, J.A. Nelson, A.C. Shabica, and C.R. Scholz, *J. Am. Chem. Soc.*, **74**, 5506 (1952).
8) A. Cave, F.X. Jarreau, Khuong-Huu-Qui, M. Leboeuf, N. Serban, and R. Goutarel, *Bull. Soc. Chim. France*, **1967**, 701.

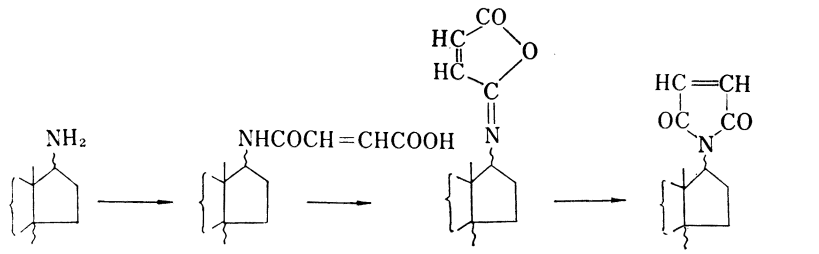


I IIa : R=H IIb : R=Ts III

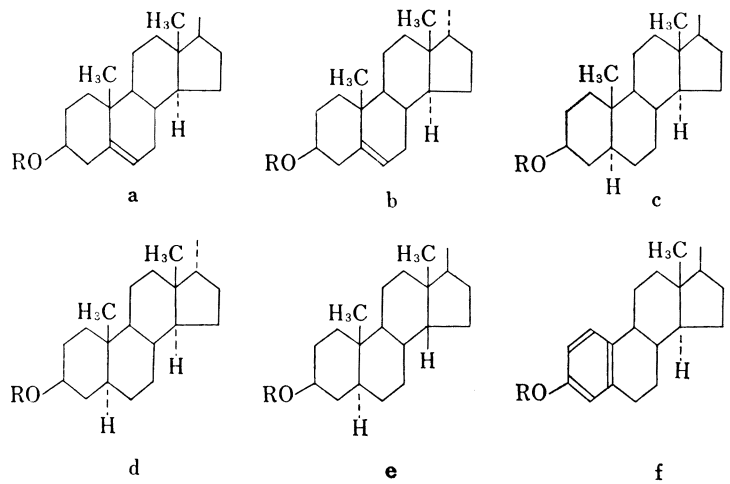
Ac=CH_3CO- , Ts=*p*-$CH_3C_6H_4SO_2-$

Chart 1

Formation of the maleimide ring was then undertaken in the usual manner[9] employing the corresponding aminosteroid (IV). Condensation with maleic anhydride proceeded with ease to provide N-steroidal maleamic acid (V) almost quantitatively. When V was heated with acetic anhydride and anhydrous sodium acetate for a prolonged time, the desired N-(steroid-17-yl)-maleimide (VII) was obtained in *ca.* 50% yield.



IV (R=H) V (R=H) VI (R=CH_3CO-) VII (R=CH_3CO-)



a b c

d e f

Chart 2

9) K.-C. Tsou, R.J. Barrnett, and A.M. Seligman, *J. Am. Chem. Soc.*, **77**, 4613 (1955); W.R. Roderick, *ibid.*, **79**, 1710 (1957).

In some cases formation of N-(steroid-17-yl)-isomaleimide (VI) was found to be successful, when N-substituted maleamic acid was heated at 100° for a short period. The resulting product was rather unstable and was transformed into maleimide derivative upon further heating. This facile isomerization of the isomaleimide was demonstrated with N-(3β-acetoxy-5α-androstan-17α-yl) derivative. The structure of isomaleimide could evidently be differentiated from that of maleimide on the basis of the nuclear magnetic resonance (NMR) spectra. In the former two olefinic protons appeared at 6.55 and 7.20 ppm as two doublets (J=6 cps), whereas in the latter both olefinic protons were equivalent and exhibited a single peak at 6.60 ppm.

The results of the biological examination on these compounds will be reported elsewhere in near future.

Experimental[10]

5α,14β-Androstane-3β,17α-diol 3-Acetate (IIa)——To a solution of 3β-acetoxy-5α,14β-androstan-17-one (310 mg) in tetrahydrofuran (20 ml) was added portionwise $NaBH_4$ (1.3 g) and the resulting solution was allowed to stand at 0° for 2.5 hr. The excess of the reagent was decomposed with AcOH and the reaction mixture was extracted with ether, washed with cold 5% $NaHCO_3$ and H_2O and dried over anhydrous Na_2SO_4. After usual work-up an oily product obtained was submitted to preparative TLC using benzene–ether (3:1) as developing solvent. Elution of the adsorbent corresponding to the spot (Rf 0.30) with acetone and recrystallization of the eluate from acetone–hexane gave IIa (160 mg) as colorless plates. mp 90—93°. $[\alpha]_D^{18}$+16.7° (c=0.12). *Anal.* Calcd. for $C_{21}H_{34}O_3$: C, 75.40; H, 10.25. Found: C, 75.02; H, 10.34.

5α,14β-Androstane-3β,17α-diol 3-Acetate 17-Tosylate (IIb)——To a solution of IIa (500 mg) in pyridine (15 ml) was added p-TsCl (600 mg) under ice-cooling and the reaction mixture was stirred at room temperature for 4 days. The resulting solution was poured onto ice-water and extracted with ether. The organic layer was washed with 5% HCl, H_2O anddried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was recrystallized from acetone to give IIb (600 mg) as colorless plates. mp 178—179°. $[\alpha]_D^{18}$+23.0° (c=0.13). *Anal.* Calcd. for $C_{28}H_{40}O_5S$: C, 68.83; H, 8.25. Found: C, 68.69; H, 8.37. NMR (5% solution in $CDCl_3$) δ: 0.78 (3H, s, 19-H), 0.89 (3H, s, 18-H), 2.02 (3H, s, 3β-$OCOCH_3$), 2.45 (3H, s, -C_6H_5-CH_3), 4.42 (1H, t, J=7 cps, 17β-H), 4.65 (1H, m, 3α-H), 7.32 and 7.78 (4H, d, J=8 cps, aromatic H).

17β-Amino-5α,14β-androstan-3β-ol (IVe)——To a solution of IIb (200 mg) in hexamethylphosphoric triamide (5 ml) was added NaN_3 (200 mg) and heated at 120° for 2 hr. The reaction mixture was diluted with ether and washed with H_2O and dried over anhydrous Na_2SO_4. On usual work-up an oily product, which consisted of 17β-azido-5α, 14β-androstan-3β-ol acetate (III) and 5α, 14β-androst-16-en-3β-ol acetate, was obtained. To a solution of this mixture in dry ether (5 ml) was added portionwise $LiAlH_4$ (400 mg) and the resulting solution was allowed to stand at room temperature for 3 hr. The excess of the reagent was decomposed with moistened ether and the reaction mixture was acidified with 10% HCl. After removal of the neutral product by extraction with ether the aq. layer was made alkaline with 10% NaOH and extracted with CH_2Cl_2. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was recrystallized from aq. MeOH to give IVe (120 mg) as colorless needles. mp 138—140°. $[\alpha]_D^{18}$+40.6° (c=0.32). *Anal.* Calcd. for $C_{19}H_{33}ON$: C, 78.29; H, 11.41; N, 4.81. Found: C, 77.93; H, 11.40; N, 4.98. NMR (5% solution in $CDCl_3$) δ: 0.79 (3H, s, 19-H), 0.97 (3H, s, 18-H), 2.84 (1H, d, J=6 cps, 17α-H), 3.55 (1H, m, 3α-H).

17β-Aminoandrost-5-en-3β-ol (IVa)——Prepared by the method of Tanabe, *et al.*[11] and recrystallized from AcOEt. Colorless needles, mp 161—162°.

17α-Aminoandrost-5-en-3β-ol (IVb)——Prepared by the method of Robinson, *et al.*[12] and recrystallized from AcOEt. Colorless needles, mp 192—194°.

17β-Amino-5α-androstan-3β-ol (IVc)——Prepared by the method of Tanabe, *et al.*[11] and recrystallized from AcOEt. Colorless needles, mp 165—167°.

10) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were determined in $CHCl_3$ solution unless otherwise specified. NMR spectra were obtained on Hitachi Model H-60 Spectrometer at 60 Mc; the chemical shifts are quoted as ppm downfield from $(CH_3)_4Si$ used as an internal standard. For preparative thin-layer chromatography (TLC) Silica gel H (E. Merck AG) was used as adsorbent.

11) S. Tanabe and M. Onda, *Yakugaku Zasshi*, **72**, 944 (1952).

12) C.H. Robinson and C. Ermann, *Steroids*, **6**, 509 (1965).

17α-Amino-5α-androstan-3β-ol (IVd)——Prepared by the method of Davis, *et al.*[13] and recrystallized from AcOEt. Colorless needles, mp 152—154°.

17β-Aminoestra-1,3,5(10)-trien-3-ol (IVf)——Prepared by the method of Tanabe, *et al.*[11] and recrystallized from AcOEt. Colorless powder, mp 219—222°.

N-(Steroid-17-yl)-maleamic Acid (V)——To a stirred solution of 17-aminosteroid (IVa—f) (1 mmole) in $CHCl_3$ (10 ml) was added dropwise a solution of maleic anhydride (1.2 mmole) in $CHCl_3$ (12 ml) at 0° and the resulting precipitate was collected by filtration and recrystallized. Yield, *ca.* 98%.

N-(Steroid-17-yl)-isomaleimide (VIa—d)——A solution of Va—d (1 mmole) in Ac_2O (5 ml) was heated with anhydrous AcONa (2 mmole) at 100° for 1 hr. The precipitate was collected by filtration, washed with H_2O and recrystallized from benzene–hexane. Yield, *ca.* 90%.

Table I

Compound	Appearance	mp (°C)	$[\alpha]_D^{MeOH}$	Formula	Analysis					
					Calcd.			Found		
					C	H	N	C	H	N
Va	colorless needles (EtOH)	249—252	−156.9° (*c*=0.12)	$C_{23}H_{33}O_4N$	71.29	8.58	3.61	71.29	8.74	3.71
Vb	colorless needles (EtOH)	195—197	+ 15.5° (*c*=0.16)	$C_{23}H_{33}O_4N \cdot \frac{1}{2}H_2O$	69.67	8.64	3.53	69.24	9.12	3.30
Vc	colorless needles (EtOH)	245—252	− 57.9° (*c*=0.11)	$C_{23}H_{35}O_4N$	70.92	9.06	3.71	70.43	8.96	3.58
Vd	colorless needles (EtOH)	218—219	+144.1° (*c*=0.10)	$C_{23}H_{35}O_4N \cdot \frac{1}{2}H_2O$	69.31	9.11	3.51	69.44	9.44	3.41
Ve	colorless prisms ($CHCl_3$–MeOH)	140—142	− 16.7° (*c*=0.12)	$C_{23}H_{35}O_4N \cdot \frac{1}{2}H_2O$	69.31	9.11	3.51	69.19	9.25	3.72
Vf	colorless needles (MeOH)	218—223	+ 59.3° (*c*=0.12)	$C_{22}H_{27}O_4N$	71.52	7.37	3.79	71.69	7.02	4.02

Table II

Compound	Appearance	mp (°C)	$[\alpha]_D^{CHCl_3}$	Formula	Analysis					
					Calcd.			Found		
					C	H	N	C	H	N
VIa	colorless prisms	211—212	−159.7° (*c*=0.15)	$C_{25}H_{33}O_4N$	72.96	8.08	3.40	72.79	7.98	3.49
VIb	colorless leaflets	228—229	− 14.0° (*c*=0.14)	$C_{25}H_{33}O_4N$	72.96	8.08	3.40	72.92	7.90	3.61
VIc	colorless prisms	207—208	− 83.5° (*c*=0.16)	$C_{25}H_{35}O_4N$	72.60	8.53	3.39	72.20	8.58	3.54
VId	colorless leaflets	217—218	+ 79.1° (*c*=0.12)	$C_{25}H_{35}O_4N$	72.60	8.53	3.39	72.69	8.59	3.71

N-(Steroid-17-yl)-maleimide (VIIa—f)——A solution of Va—f (1 mmole) in Ac_2O (5 ml) was heated with anhydrous AcONa (1.6 mmole) at 120° for 10 hr. The resulting solution was poured onto ice–water and stirred to decompose the excess of the reagent. The precipitate was collected by filtration, washed with H_2O and dried. The crude product was purified by preparative TLC and recrystallized. Yield, *ca.* 50%.

Transformation of N-(3β-Acetoxy-5α-androstan-17α-yl)-isomaleimide (VId) into N-(3β-Acetoxy-5α-androstan-17α-yl)-maleimide (VIId)——A solution of VId (100 mg) in Ac_2O (3 ml) was heated with anhydrous AcONa (35 mg) at 120° for 10 hr. The resulting solution was poured onto ice–water and stirred to decompose the excess of the reagent. The precipitate was collected by filtration, washed with H_2O and dried. The crude product was submitted to preparative TLC using benzene–ether (10:1) as developing solvent. Recrystallization of the eluate from benzene–hexane gave VIId as colorless needles, mp 201—

13) M. Davis, E.W. Parnell, and D. Warburton, *J. Chem. Soc.* (*C*), **1966**, 1698.

Table III

Compound	Appearance	mp (°C)	$[\alpha]_D^{CHCl_3}$	Formula	Analysis					
					Calcd.			Found		
					C	H	N	C	H	N
VIIa	colorless needles ($CHCl_3$–hexane)	265—267	−100.0° (c=0.10)	$C_{25}H_{33}O_4N$	72.96	8.08	3.40	72.66	8.11	3.25
VIIb	colorless needles (benzene–hexane)	196—198	− 5.0° (c=0.10)	$C_{25}H_{33}O_4N$	72.96	8.08	3.40	73.12	8.32	3.61
VIIc	colorless needles ($CHCl_3$–hexane)	252—253	+ 43.6° (c=0.11)	$C_{25}H_{35}O_4N$	72.60	8.53	3.39	72.59	8.65	3.71
VIId	colorless needles (benzene–hexane)	201—202	+ 33.1° (c=0.11)	$C_{25}H_{35}O_4N$	72.60	8.53	3.39	72.20	8.68	3.74
VIIe	colorless needles ($CHCl_3$–hexane)	203—205	0° (c=0.12)	$C_{25}H_{35}O_4N$	72.60	8.53	3.39	72.40	8.72	3.71
VIIf	colorless needles (ether–hexane)	145—147	− 24.3° (c=0.10)	$C_{24}H_{27}O_4N$	73.26	6.92	3.56	73.69	7.02	3.48

202°. Yield, 53 mg. Mixed melting point on admixture with the sample derived from N-(3β-hydroxy-5α-androstan-17α-yl)-maleamic acid (Vd) showed no depression and infrared spectra of two samples were entirely identical in every respect.

Acknowledgement The authors express their thanks to Misses Michiko Wada and Lai Yin Ng for their skillful technical assistance. They are also indebted to all the staffs of central analytical laboratory of this Institute for elemental analyses and spectral measurements. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, which is gratefully acknowledged.