and an unidentified aliphatic unsaturated hydrocarbon (b.p. 78–80 °C).

According to SANDRIS and OURISSON[2] it was possible to obtain the 1-benzoyl-2, 2, 5, 5-tetramethyl-3-pyrrolidinone (II) from 2, 2, 5, 5-tetramethyl-3-pirrolidinone (I), using benzene, in the presence of triethylamine (Yield 79%, b.p. 143–145 °C/0.25 mm Hg, mp 55–57 °C. Anal. Calc. for $C_{15}H_{19}NO_2$ (245.3) C 73.44 H 7.80 *N* 5.71, Found C 72.93 H 7.68 *N* 5.69).

$$\nu_{CO\ ketone} = 1758\ cm^{-1};\quad \nu_{CO\ amide} = 1628\ cm^{-1}.$$

1-benzoyl-2, 2, 5, 5-tetramethyl-3, 4-pyrrolidindione (III) was prepared by oxidation of (II) using SeO_2, in aqueous dioxane, at 80 °C (Yield 58%[6], mp 112–114 °C; Anal. Calc. for $C_{15}H_{17}NO_3$ (259.3) C 69.48 H 6.60 *N* 5.40, Found C 68.88 H 6.69 *N* 5.37).

$$\nu_{CO\ ketone} = 1782\text{–}1769\ cm^{-1};\quad \nu_{CO\ amide} = 1640\ cm^{-1}.$$

1-benzoyl-3-hydroxy-2, 2, 4, 4-tetramethyl-3-azetidincarboxylic acid (V) was obtained by heating the compound (III) in aqueous solution of KOH 20%, until boiling. (Yield 91%, mp 253–255 °C (dec.); Anal. Calc. for $C_{15}H_{19}NO_4$ (277.3) C 64.97 H 6.60 *N* 5.05, Found C 65.32 H 7.01 *N* 5.05).

$$\nu_{OH} = 3419\ cm^{-1};\quad \nu_{CO\ ketone} = 1690\ cm^{-1};$$
$$\nu_{CO\ amide} = 1550\ cm^{-1}.$$

1-benzoyl-2, 2, 4, 4-tetramethyl-3-azetidinone (VII) was prepared by oxidation of (V) with $Pb(CH_3COO)_4$ in $CHCl_3$ (Yield 97%, b.p. 120–123 °C/0.4 mm Hg; mp 61–63 °C; Anal. Calc. for $C_{14}H_{17}NO_2$ (231.3) C 72.69 H 7.40 *N* 6.05, Found C 73.11 H 7.38 *N* 6.07).

$$\nu_{CO\ ketone} = 1825\ cm^{-1};\quad \nu_{CO\ amide}\ 1625\ cm^{-1}.$$

1-benzoyl-2, 2, 4, 4-tetramethyl-azetidine (IX) was obtained by a method similar to that used for (VI) (Yield 64%, mp 103–105 °C; Anal. Calc. for $C_{14}H_{19}NO$ (217.3) C 77.49 H 8.82 *N* 6.45; Found C 76.36 H 8.68 *N* 6.48).

$$\nu_{CO\ amide} = 1620\ cm^{-1}.$$

1-benzyl-2, 2, 4, 4-tetramethyl-azetidine (X) prepared by reduction of (IX) with $LiAlH_4$, in ethyl ether, was isolated, as the chlorhydrate. (Yield 96%, mp 174–176 °C; Anal. Calc. for $C_{14}H_{21}N \cdot HCl$ (239.8) C 70.06 H 9.40 *N* 5.83 Cl 14.77. Found C 70.54 H 9.38 *N* 5.84 Cl 14.63).

2, 2, 4, 4-tetramethyl-azetidine (XI) was obtained by hydrogenolysis of (X). HCl, in ethanol, in the presence of Pd/C 10%, and isolated as chlorhydrate. (Yield 82%, mp 198–200 °C; Anal. Calc. for $C_7H_{15}N \cdot HCl$ (149.6) C 56.20 H 10.78 *N* 9.36 Cl 23.68; Found C 56.41 H 10.66 *N* 9.38 Cl 23.65).

Chemical shifts, ppm (τ)[a]

		ϕ	$-CH_3$	$-CH_2$	-NH-	-OH-
II	CCl_4	2.70 s	8.50[b] s 8.72[c] s	7.5 s	–	–
III	CCl_4	2.68 s	8.52[d] s	–	–	–
V	DMSO(d_6)	2.60 s	8.35 b 8.78 b	–	–	3.00[e] b
VII	CCl_4	2.62 s	8.52 s	–	–	–
IX	CCl_4	2.69 s	8.60 b	8.10 s	–	–
X	CCl_4	2.85 m	8.90 s	6.45[f] s 8.30[g] s	–	–
XI	CCl_4	–	8.80 s	8.10 s	7.53[h] s	–

[a] The NMR-spectra were recorded on Perkin Elmer R 12, at 60 MHz. [b] 6 protons of methyl groups vicinal to –CO– function. [c] 6 protons of methyl groups vicinal to $-CH_2-$. [d] 12 protons of methyl groups vicinal to –CO– function. [e] The assignement is uncertain, but the structure of the compound was confirmed by IR-spectra. [f] $-CH_2-$ of benzylic group. [g] $-CH_2-$ of heterocyclic ring. [h] This signal is removed completely when few drops of D_2O are added to CCl_4. s, single; m, multiple; b, very broad peak.

Riassunto. La 2, 2, 4, 4-tetrametil-azetidina (XI), è stata preparata per la prima volta con ottime rese, a partire da 2, 2, 5, 5-tetrametil-3-pirrolidone (I), attraverso i seguenti intermedi: 1-benzoil-2, 2, 5, 5-tetrametil-3-pirrolidone (II), 1-benzoil-2, 2, 5, 5-tetrametil-3, 4-pirrolidindione (III), acido 1-benzoil-3-idrossi-2, 2, 4, 4-tetrametil-3-azetidin-carbossilico (V), 1-benzoil-2, 2, 4, 4-tetrametil-3-azetidinone (VII), 1-benzoil-2, 2, 4, 4-tetrametil-azetidina (IX) e 1-benzil-2, 2, 4, 4-tetrametil-azetidina (X).

L. DALL'ASTA and A. PEDRAZZOLI[7]

Research Departement 'Midy',
Via Piranesi 38, Milano (Italy), 16 April 1970.

[7] The authours gratefully acknowledge the collaboration of Dr. G. MAFFI for the interpretation of the IR-spectra, of Dr. A. DEGLI ANGELI for the interpretation of the NMR-spectra and of Mr. E. GAREGNANI for technical assistance.

Modification of Digitalis Inotropism by a Lactam Derivative

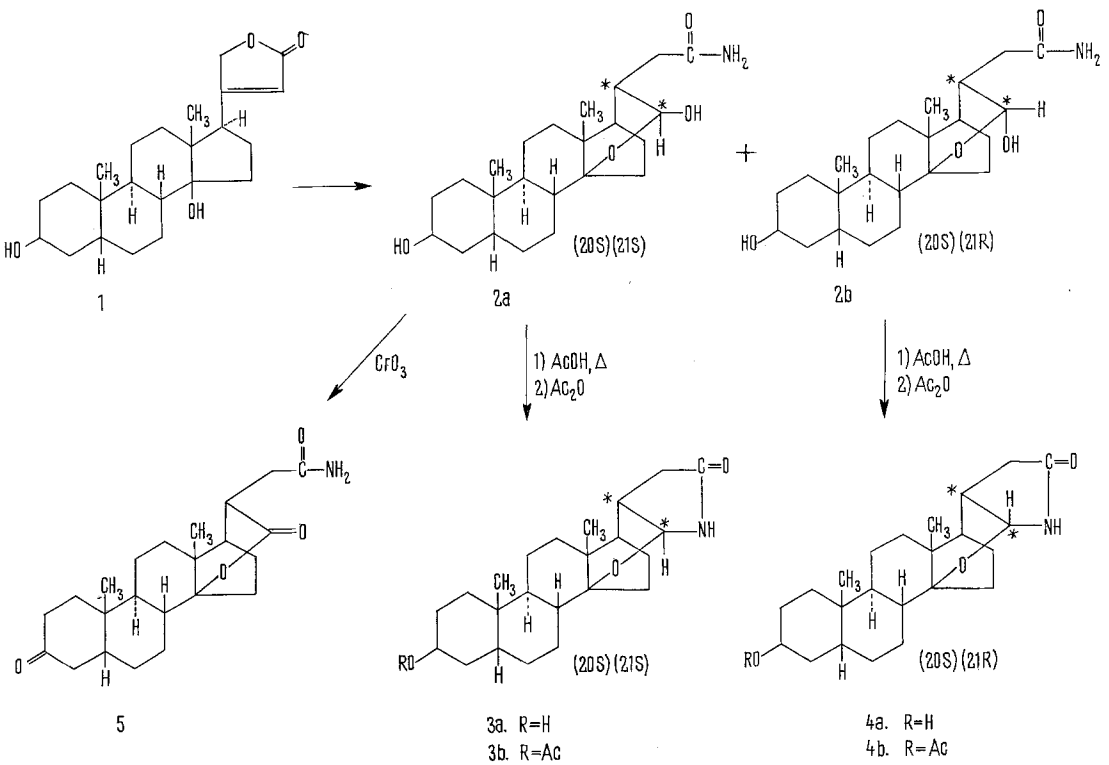
The importance of the unsaturated lactone ring for typical effects of cardiotonic steroids has been well documented[1, 2]. Recently, derivatives of such steroids have been synthesized with specific changes in the group located at the C-17 position[3–7]. One of these compounds, acetylisodigitoxigeninic lactam, reduces the effects of a subsequent dose of a standard cardenolide lactone.

Ammonolysis of digitoxigenin **1** in methanol solution affords lactol amide **2a**[8] (55%) mp 271–273°, $[\alpha]_D^{20}$ – 5° (*c* 1, pyridine) and lactol **2b**, mp 201–203°, $[\alpha]_D^{20}$, +44° (*c*, 1, pyridine), concomitantly formed in 14% yield. That **2a** and **2b** differ only in the orientation of the hydroxyl group at C-21 is shown by oxidation (CrO_3-pyridine) of either lactol to the same lactone **5**, mp 284–286°, $[\alpha]_D^{20}$ – 3°

(*c*, 1, pyridine). Molecular models show that the lactol rings of these compounds assume the more stable chair conformation and that in the most stable isomer (**2a**), the amide chain at C-20 and the hydroxyl group at C-21 are *trans*-diequatorial. In the second, less stable, isomer (**2b**), the amide chain at C-20 is still equatorial, but the hydroxyl group at C-21 is axial. Heating **2a** to 200° or treatment with warm glacial acetic acid causes rapid cyclization to lactam **3a**, mp 275–277°, $[\alpha]_D^{20}$ — 19° (*c*, 1, pyridine). Acetylation of **3a** (Ac_2O-pyridine) yields acetate **3b**, mp 273–275°, $[\alpha]_D^{20}$ — 39° (*c*, 1, chloroform). Similar treatment of lactol **2b** gives the epimeric lactam **4a**, mp 266–268°, $[\alpha]_D^{20}$ — 62° (*c*, 1, pyridine). Acetylation as before yields acetate **4b**, mp 241–243°, $[\alpha]_D^{20}$ — 25° (*c*, 1, chloroform). We have given **4b** the trivial name acetyl-isodigitoxigeninic lactam. Lactol **2a** cyclizes to give diequatorially fused lactam **3a**, whereas lactol **2b** cyclizes to form the equatorial-axial lactam **4a**. In support of this, lactam **3a** is stable in warm, dilute solution of aqueous dioxane containing 0.5% of *p*-toluenesulfonic acid, whereas lactam **4a** under the same conditions opens to form lactol **2b** with partial concomitant isomerization to the more stable lactol **2a**. (20 S, 21 S)-3β-Hydroxy-21-amino-14β, 21-oxidonorcholan-23-oic acid lactam tridigitoxoside (trioside of **3a**, mp 269–272°, was prepared from digitoxin by ammonolysis in methanol solution followed by cyclization in acetic acid. The configuration at C-21 was deduced from the NMR-spectrum[9].

Isometric contractility of isolated guinea-pig and rabbit left atria was studied using a positive inotropic lactone, 3-acetyldigitoxigenin, and the lactam (**4b**). Because the duration of lactam effects is short, we chose a standard dose of acetyldigitoxigenin which produced a large inotropic response in the first 10 min and excluded muscles (about 10%) which showed toxic effects during the first 20 min of exposure. The results are shown in Table I. The degree of inhibition, calculated as a percentage ,is shown in Table II. Compound **3b** and its tridigitoxyl glycoside appeared devoid of inhibitory effects in preliminary experiments. Other experiments suggested that increasing the dose of lactone decreases the degree of inhibition produced by a given concentration of lactam. The lactam ($1 \times 10^{-4} M$) had no effect on the inotropic response to increasing the Ca^{++} concentration from 2 to 4 mM (2 muscles). In preliminary experiments in cats, the lactam had no effect on the EKG and did not influence the toxic effects of the lactone.

Lubrol extracts containing Na^+-K^+-ATPase activity were prepared from guinea-pig cardiac microsomal fractions[10]. The lactam at concentrations between 10^{-6} and



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[8] Satisfactory elemental analyses were obtained for all new compounds. Confirmatory high resolution mass spectra were determined on compound *3b*, *4b*, and *5*. Structures assigned are consistent with NMR-spectra and homogeneity was established by TLC. Melting points were obtained using a corrected thermometer.
[9] The preparation of *3a*, *3b* and *4b* in another way has been claimed in a recent patent (J.-H. FERLAND and Y. LEFEBVRE, US-Patent 3,462,413 through Chem. Abstr. *71*, 113228 (1969) but the melting points differ widely from those reported here.
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$10^{-4}M$ had no significant effect on Na^+-K^+-ATPase activity and did not influence the inhibitory action of the lactone when both compounds were added to the reaction media at the same time (Table III). In other experiments, preparations were preincubated for 10 min with the lactam ($10^{-4}M$) before addition of the lactone. The degree of lactone inhibition (60%) of the Na^+-K^+-ATPase activity was the same whether or not the lactam was present. In similar experiments using lubrol extracts of rat brain microsomal fractions[10] and conventional rat cardiac microsomal preparations[11] the lactam had no effect on Na^+-K^+-ATPase activities and no influence on the sensitivity of such preparations to either 3-acetyldigitoxigenin or ouabain.

Table I. Isometric contractile force of guinea-pig left atria

Conditions	Time (min)				
	10 (%)	13 (%)	15 (%)	20 (%)	30 (%)
A. Lactone	98 ± 4	129 ± 7	147 ± 8	185 ± 14	243 ± 25
B. Lactam + lactone	94 ± 5	103 ± 5	115 ± 5	151 ± 8	214 ± 17
C. Lactam	108 ± 4	–	–	96 ± 5	83 ± 5
D. No treatment	94 ± 2	–	–	89 ± 4	84 ± 4
E. DMSO	98 ± 3	–	–	–	–

Muscles were stimulated at 1/sec in Krebs-Henseleit solution containing 2 mM Ca^+ at 35.5 °C. Dimethylsulfoxide (DMSO) 0.42 ml or lactam ($1 \times 10^{-4}M$ final concentration) in 0.42 ml DMSO was added to the 50 ml bath at time zero. 3-acetyldigitoxigenin ($3 \times 10^{-6}M$ final concentration) in 12.5 µl DMSO was added at 10 min. Data are expressed as percent of contractility at zero time ± standard error of the mean. Each condition was studied in each muscle and the order of treatments was systematically varied ($N = 6$).

Table II. Inhibition of guinea-pig left atrial inotropic response to lactone ($3 \times 10^{-6}M$) caused by prior administration of lactam ($1 \times 10^{-4}M$)

Time (min)	Statistic		
	Mean inhibition (%)	SEM (%)	Probability
13	87.2	7.4	< 0.001
15	80.3	6.6	< 0.001
20	35.7	11.1	< 0.02
25	19.2	13.6	> 0.1
30	11.2	14.0	> 0.1

Lactam added at zero time, lactone at 10 min. The percentage inhibition was calculated for each muscle and then averaged. SEM, standard error of the mean. Probability of no significant inhibition (null hypothesis) was calculated by student t-test ($N = 6$).

Table III. Sensitivity of Na^+-K^+ ATPase preparations of guinea-pig heart to lactone and lactam

Conditions	Na^+-K^+-ATPase activity (µmoles Pi/mg protein/h)	Inhibition (%)
Control	20.3 ± 1.6	–
Lactone ($10^{-5}M$)	7.3 ± 0.5	64.4
Lactam ($10^{-4}M$)	19.8 ± 1.0	3.1
Lactone ($10^{-5}M$) + lactam ($10^{-4}M$)	6.8 ± 0.1	64.9

Total Na^+-K^+-Mg^{++}-ATPase activity was measured by conventional orthophosphate release methods[11] during incubation at 37 °C for 10 min. Mg^{++}-ATPase activity determined in the absence of Na^+ and K^+ gave values for Na^+-K^+-ATPase by subtraction.

Discussion. The physiological experiments suggest that the lactam can delay or inhibit cardenolide (lactone) inotropism. Other agents which have been reported to delay or inhibit cardenolide inotropism include tetrodotoxin[12], aldosterone[13], and manganese[14]. In the presence of the lactam, the inhibitory effect of 3-acetyldigitoxigenin on Na^+-K^+-ATPase system is unimpaired while the early inotropic action is markedly reduced. These data and the lack of effect of the lactam on the toxic EKG manifestations of subsequent doses of the lactone are consistent with the hypothesis that the toxic effect of cardenolides may be related to inhibition of cation transport ATPase[2]. The data also suggest that digitalis steroids may produce Na^+-K^+-ATPase inhibition in isolated myocardium without a concurrent positive inotropic response.

The primary effect of the lactam may be to delay the onset rather than to inhibit the final inotropic action of the lactone. DUTTA et al.[15] have postulated a mechanism responsible for transport of cardiotonic steroids across the cell membrane. WASSERMAN and HOLLAND[12] showed that tetrodotoxin can produce a delay of onset of ouabain inotropism. These reports and our evidence support the concept of a carrier mechanism for cardiotonic steroids which may be linked to passive and/or active cation flux. Inhibition of such a mechanism by the lactam could prolong the time required for cardenolides to reach optimal concentrations at the inotropic receptor[16].

Note added in proof: Recent experiments show that acetylisodigitoxigenin, like acetylisodigitoxigeninic lactam significantly inhibits the early inotropic response of guinea-pig atria to cardenolides.

Resumen. Se describe la síntesis y prueba de estructura del 3-acetato de (20 S, 21 R)-3β-hidroxi-21-amino-14β, 21-oxidonorcolan-23-oico ácido lactama, el cúal es el derivativo lactama de digitoxigenina. Dicho compuesto posée insignificantes efectos inotrópicos, pero inhibe o retarda la acción inotrópica positiva del 3-acetato de digitoxigenina cuando se ensaya en atria izquierda aislada de cochinillo de Indias o conejo.

B. G. KATZUNG, J. A. MUNOZ, D. Y. SHIRACHI, A. J. TREVOR, H. H. CHANG and M. E. WOLFF

University of California San Francisco, Department of Pharmacology, San Francisco (California 94122, USA), 2 June 1970.

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