KETOIMINES OF CARDENOLIDES

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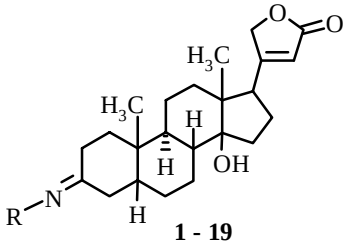
*3-Keto-derivatives of the cardenolides digitoxigenin and cardiogenin were prepared with subsequent conversion to new ketoimines (**1-19**, **24-44**), the oxime (**23**), and for the first time to pure cardiogenin (14,16-dianhydrogitoxigenin, **20**), its acetate (**21**), and cardiogenone (**22**). Their physicochemical properties were described.*

Key words: cardenolides, digitoxigenin, imines, digitoxigenone, digitoxigenone-ketoimines, gitoxigenin, cardiogenone, 14,19-dianhydrogitoxigenin, cardiogenone, cardiogenone-ketoimines.

The chemistry of cardenolide ketoimines is a poorly studied area of steroidal cardiotonics. This is due mainly to the fact that ketocardenolides are unknown in nature, in contrast with the widely distributed natural aldehydocardenolides. Ketocardenolides became available when the very convenient oxidant for labile compounds, Sarett reagent, which is a complex of chromic anhydride and pyridine [1], began to be used to oxidize the secondary OH groups in natural compounds. This reagent is described in modified terms in the literature [2] and in the Experimental section of the present article.



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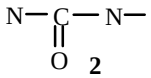
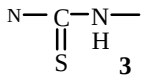
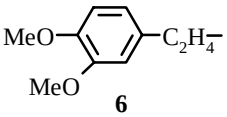
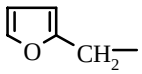
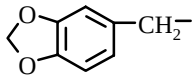
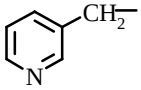
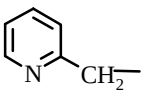
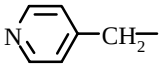
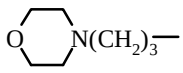
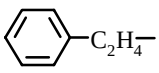
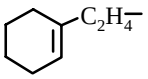
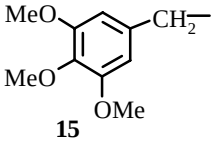
R = C_6H_5-NH– **1**;  **2**;  **3**; $C_6H_5CH_2$– **4**; $CH_3CH_2CH_2CH_2$– **5**

 **6**;  **7**;  **8**;  **9**;  **10**;  **11**

 **12**;  **13**;  **14**;  **15**; $CH_3(CH_2)_9$– **16**; $CH_3(CH_2)_{11}$– **17**

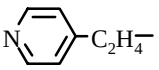
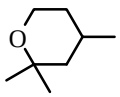
 **18**;  **19**

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TABLE 1. Physicochemical Data for **1**-**19** and **23**-**44**

Compound	Molecular formula	mp, °C	$[\alpha]^{20}_D$, deg, EtOH	Compound	Molecular formula	mp, °C	$[\alpha]^{20}_D$, deg, EtOH
1	$C_{29}H_{38}N_2O_3$	122-125/143-146	+60.0±3	**25**	$C_{24}H_{31}N_3O_3$	200-203/237-240	+731.1±5
2	$C_{24}H_{35}N_3O_4$	205-209/235-237	+40.4±4	**26**	$C_{24}H_{31}N_3O_2S$	210-214/335-339	+700.9±5
3	$C_{24}H_{35}N_3O_3S$	183-187/205-208	+57.2±4	**27**	$C_{29}H_{39}NO_2$	101-103/109-112	+513.3±5
4	$C_{30}H_{39}NO_3$	94-97	+38.4±2	**28**	$C_{30}H_{35}NO_2$	94-96	+563.8±5
5	$C_{27}H_{41}NO_3$	95-97	+34.3±2	**29**	$C_{27}H_{37}NO_2$	102-105	568.7±5
6	$C_{33}H_{45}NO_5$	Amorph.	+30.1±2	**30**	$C_{33}H_{41}NO_2$		+399.1±5
7	$C_{29}H_{37}NO_4$	90-93	+29.8±3	**31**	$C_{28}H_{33}NO_3$	93-97	+469.3±5
8	$C_{31}H_{39}NO_5$	Amorph.	+39.6±3	**32**	$C_{31}H_{35}NO_4$		+418.8±5
9	$C_{29}H_{38}N_2O_3$	Amorph.	+31.3±3	**33**	$C_{29}H_{34}N_2O_2$	94-97	+458.0±5
10	$C_{29}H_{38}N_2O_3$	202-205	+33.9±2	**34**	$C_{29}H_{34}N_2O_2$	93-97	+477.5±5
11	$C_{29}H_{38}N_2O_3$	91-95	+29.3±3	**35**	$C_{29}H_{34}N_2O_2$	99-103	+522.1±5
12	$C_{30}H_{46}N_2O_4$	Amorph.	+29.6±2	**36**	$C_{30}H_{42}N_2O_3$	180-182/192-193	+479.2±10
13	$C_{31}H_{41}NO_3$	Amorph.	+38.7±3	**37**	$C_{28}H_{39}N_3O_2$	102-104/115-117	+564.0±5
14	$C_{31}H_{45}NO_3$	91-93	+28.6±3	**38**	$C_{31}H_{41}NO_2$	89-92	+418.9±5
15	$C_{33}H_{45}NO_6$	90-93	+38.4±3	**39**	$C_{31}H_{39}NO_3$	195-198	+277.0±5**
16	$C_{33}H_{53}NO_3$	97-102	+23.5±2	**40**	$C_{33}H_{49}NO_2$	185-190	+575.4±5**
17	$C_{35}H_{57}NO_3$	102-105	+24.1±3	**41**	$C_{30}H_{37}NO_2$	98-101	+515.3±5**
18	$C_{30}H_{46}N_2O_3$	185-189	+30.2±2	**42**	$C_{33}H_{41}NO_5$	115-118	+491.5±5**
19	$C_{30}H_{45}NO_4$	103-104	+31.5±2	**43**	$C_{35}H_{53}NO_2$	110-114/169-172	+442.0±5**
23	$C_{23}H_{29}NO_3$	145-149	+698.9±10	**44**	$C_{27}H_{36}N_2O_3$	115-118/155-159	+608.9±5**
24	$C_{29}H_{34}N_2O_2$	155-158/192-195	+702.3±5*				

*$CHCl_3$; **CH_3OH.

We found data in the literature for only two synthesized N-derivatives of digitoxigenone of similar structure: guanidylhydrazone and digitoxigenone S-methyl-*iso*-thiosemicarbazone [3].

The starting natural compounds for synthesizing **1**-**44** were digitoxigenin [4] and cardiogenin [4, 5], which were obtained from the corresponding natural glycosides. Digitoxigenin was prepared by hydrolysis from digitoxin or from lanatoside A; cardiogenin (14,16-dianhydrogitoxigenin), from gitoxin. Cardiogenin was prepared by combining into one step, as described below, hydrolysis of gitoxin to gitoxigenin [4] and final cleavage of the C-14 and C-16 OH groups.

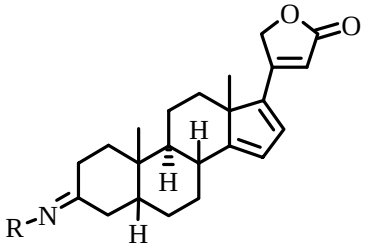
Compounds **1**-**19** and **22**-**44** were synthesized in two steps. The first was oxidation of digitoxigenin and cardiogenin with isolation of the corresponding 3-ketones. The second was preparation of the ketoimines by direct reaction of the ketones with primary amines with boiling in appropriate solvents and azeotropic removal of water released during the reaction.

The physicochemical properties of cardiogenin, with the exception of the melting point, have not been reported. It is interesting that the specific rotation has an unusually high numerical value, $[\alpha]_D^{20}$ +545±5° ($CHCl_3$). The presence in cardiogenin of a long conjugated C=C system is responsible for the intense blue luminescence in UV light. This system gives in the IR spectrum two absorption bands with maxima at 1608 and 1575 cm^{-1}. The UV spectrum has λ_{max} (MeOH) at 219 and 338 nm. 3-O-Acetylcardiogenin (**21**) and 3-ketocardiogenin (**22**) are also new and not previously descibed. Their properties are reported for the first time (see Experimental).

The IR spectrum of **22** has strong absorption bands at 1606 and 1574 cm^{-1} that belong to C=C bonds of a conjugated system and split bands with maxima at 1710 cm^{-1} (ketone) and 1650 cm^{-1} (carbonyl of the butenolide ring).

It is known that imines can exist in *syn*- and *anti*-isomers.

Apparently, in this instance this also occurs. However, the potentially possible isomers are inseparable by paper and thin-layer chromatography.



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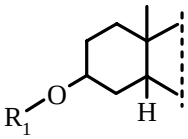

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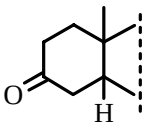
21: R_1 = Ac



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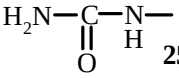
R = HO **23** C_6H_5-NH- **24**



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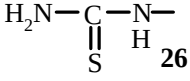

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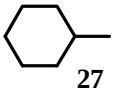
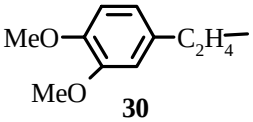


27 $C_6H_5CH_2$- **28** $CH_3(CH_2)_3$- **29**



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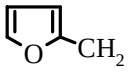

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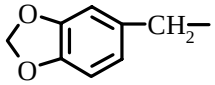

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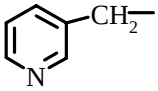

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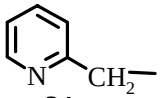

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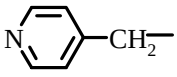

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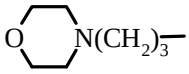

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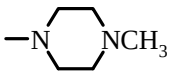

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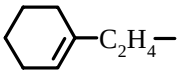

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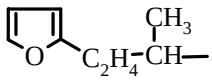

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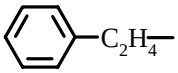
$CH_3(CH_2)_9$- **40**



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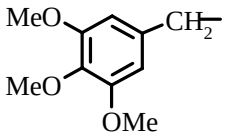

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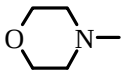

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EXPERIMENTAL

The course of reactions and purity of products were monitored using TLC on Silufol UV-254 plates with elution by $CHCl_3$:CH_3OH:H_2O (85:15:0.7). Elemental analyses were performed on a Model 1106 automated C—H—N—S analyzer. Elemental analyses of all compounds agreed with those calculated. IR spectra were recorded on a Specord-75 IR (KBr disks).

Cardiogenin (20 = $\Delta^{14,16}$-dianhydrogitoxigenin). Gitoxin (54 g) was placed into a 2-liter flask with a ground-glass joint and treated with $CHCl_3$:CH_3OH (1.5:1, 600 mL) and conc. HCl (30 mL, d = 1.179) diluted with water (25 mL). The mixture was refluxed for 9 h, cooled to room temperature, and treated with Na_2CO_3 solution (5%) until weakly basic. The aqueous layer was separated and discarded. The $CHCl_3$:CH_3OH layer was washed with water (100 mL) and evaporated to dryness to give an amorphous powder (25 g). The powder was dissolved in CH_2Cl_2 (50 mL), treated with CH_3OH (50 mL), concentrated on a hot-water bath to a CH_3OH residual (40 mL), and left at room temperature overnight. The resulting crystals were collected on a Buchner funnel, washed with CH_3OH (20 mL), and dried in air to give yellow crystals (11.3 g).

The mother liquors were evaporated to dryness. The solid was chromatographed over Al_2O_3 (400 g, grade III Brockmann activity) with elution by CCl_4:$CHCl_3$ (3:1→1:2). Fractions (40 mL) were collected using an automated collector. Fractions 8-29 were the purest and were combined and evaporated. The product crystallized from CH_3OH as described above to give additional cardiogenin (10.1 g; total, 21.4 g, 87% of calculated). Cardiogenin (**20**): mp 207-210°C, $[\alpha]_D^{20}$ +545.0±5° (*c* 1.0, $CHCl_3$), $C_{23}H_{30}O_3$. It fluoresces bright blue in UV light.

3-O-Acetylcardiogenin (21). Cardiogenin (**20**, 1 g) was dissolved in pyridine (3 mL), treated with acetic anhydride (1.5 mL), and left for 17 h at room temperature. The reaction mixture was transfered to a flask with ice and stirred for 30 min. The solid product (**21**) was separated on a Buchner funnel, washed with water, and dried in vacuum.

Recrystallization. The crude product was dissolved in CH_2Cl_2 (7 mL), treated with CH_3OH (30 mL), and concentrated to a CH_3OH residual (~5 mL). The resulting crystals were separated and washed with CH_3OH (4 mL) to give pure crystalline 3-O-acetylcardiogenin (**21**, 0.95 g), $C_{25}H_{32}O_4$, mp 115-117°C, $[\alpha]_D^{20}$ +555.9±5° (*c* 0.58, CH_3OH).

3-Ketocardiogenin (3-Keto-$\Delta^{14,16}$-dianhydrogitoxigenin, 22). Oxidant was prepared by mixing absolute pyridine (36 mL) with $CHCl_3$ (220 mL) that was washed with water to remove alcohol impurities, dehydrated over anhydrous Na_2SO_4, and distilled azeotropically. The solution was cooled with ice, stirred, and cooled as CrO_3 (25 g) was added. The cold mixture was stirred for 10 min and shaken in a shuttle apparatus for 30 min at room temperature.

The oxidation was carried out by dissolving cardiogenin ($\Delta^{14,16}$-dianhydrogitoxigenin, 22.4 g) in purified $CHCl_3$ (70 mL) and pyridine (25 mL). The solution was poured into a reaction flask containing the prepared oxidant. The mixture was stirred in a shuttle apparatus for 5 h. Additional oxidant was added (CrO_3, 8 g; $CHCl_3$, 30 mL; pyridine, 8 mL). After 3 h the same amount of oxidant was added. The reaction was continued for 2 h. The oxidation took 10 h.

The solution was separated from the solid by filtration through filter paper. The solid was washed with $CHCl_3$. The combined $CHCl_3$ solution was washed once with water. The water layer was extracted twice with $CHCl_3$, which was combined with the main solution. The solution was washed four times with H_2SO_4 (10%) and once with Na_2CO_3 solution (2%) (until weakly basic), dried over anhydrous Na_2SO_4, and concentrated to about 200 mL, removing traces of water.

The $CHCl_3$ solution was purified with Al_2O_3 (30 g) by shaking the suspension for 7 min, filtering, and evaporating to dryness. The solid (22 g) was dissolved in CH_2Cl_2 (70 mL), treated with CH_3OH (200 mL), and concentrated to about 150 mL. The resulting crystals were separated and washd with CH_3OH (50 mL) to give grayish crystals (17 g).

The crude product (17 g) was recrystallized by dissolving in CH_3OH (600 mL) with heating in a flask with a reflux condenser, adding Al_2O_3 (level III Brockmann activity), stirring, and vacuum filtering the hot solution through a packed layer of kieselguhr. The filtrate was concentrated (without vacuum) to ~50 mL and left overnight.

The crystals were separated, washed with CH_3OH (30 mL), and dried in air to give **22** (14 g) as white crystals with a yellow tint, $C_{23}H_{28}O_3$, mp 198-199°C, $[\alpha]_D^{20}$ +630.0±5° (*c* 1.3, CH_3OH).

Digitoxigenone. Digitoxigenin (25 g) was oxidized as described above for cardiogenin to give the final product (15 g) as white crystals, $C_{23}H_{32}O_4$, mp 160-162/191-194°C, $[\alpha]_D^{20}$ +27.6±2° (*c* 1.0, CH_3OH).

Digitoxigenone Benzylimine (6). Digitoxigenone (1.1 g) was dissolved in benzene:2-propanol (15 mL, 9:1) with heating and placed in a long-necked flask. The solution was treated with benzylamine ($C_6H_5CH_2NH_2$, 0.38 g, 1.2-fold of the calculated amount). The mixture was boiled for 2.5 h, slowly reducing its volume to about 2 mL and adding benzene three times (2 mL each), and evaporated in vacuum to dryness. The solid was a glassy mass. It was dissolved in CH_3OH (3 mL) and treated with ice. The resulting solid was ground into a powder, separated on a Buchner funnel, washed with icewater (50 mL), and dried in vacuum to give the amorphous product (1.36 g).

The amorphous powder (1.36 g) was crystallized by dissolving in CH_2Cl_2 (3 mL), adding CH_3OH (15 mL), concentrating to about 5 mL to remove completely the CH_2Cl_2, and crystallizing at room temperature (17 h). The resulting crystals were separated and washed with CH_3OH to give the final product (**6**), $C_{30}H_{39}NO_3$, mp 94-97°C, $[\alpha]_D^{20}$ +38.4±2° (*c* 1.1, C_2H_5OH).

Cardiogenone Butylimine (9). Cardiogenone (**22**, 1.1 g) was placed in a long-necked flask, dissolved in benzene (15 mL), and treated with *n*-butylamine [$CH_3(CH_2)_3NH_2$, 0.287 g, 1.2-fold of the calculated amount]. The solution was boiled for 30 min until vapor was no longer given off and dried in vacuum. The solid was dissolved with heating in 2-propanol (3.5 mL). Crystals formed from the solution at room temperature. These were separated after 17 h, washed with 2-propanol, and dried.

The final product is yellow and crystalline, $C_{27}H_{37}NO_2$, mp 102-105°C, $[\alpha]_D^{20}$ +568.7±5° (*c* 1.0, C_2H_5OH).

The other digitoxigenone and cardiogenone imines were prepared similarly. The differences were mainly in the reaction times which, as already noted, were monitored using TLC.

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