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Studies on the Synthesis of Cardiotonic Steroids. I. Efficient Synthesis of Cardenolides*

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Practical synthetic methods of natural cardenolides are illustrated in the syntheses of xysmalogenin and digitoxigenin. The new routes employ pregn-14-en-20-one as key intermediate which was prepared by partial reduction of 14,16-dien-20-one with triphenylstannane or triethylsilane. A general and efficient method was devised for obtaining cardenolides consisting of (1) 21-methylsulfenylation of pregnen-20-one, (2) the reaction of the resulting 21-methylthio derivative with bromoacetate and zinc, (3) alumina chromatography of epoxy ester obtained from the S-methylated Reformatsky product.

The naturally occurring cardenolides are well known for their specific effect upon heart muscle, and structurally they possess the normal steroid ring system with a 14β-hydroxy substituent as well as an α,β-unsaturated γ-lactone grouping at 17β-position.[2] A typical member of the group is digitoxigenin[3β,14β-dihydroxycard-20(22)-enolide], and its tri-digitoxide (digitoxin) is a constituent of the drug digitalis and has been used as a clinical medicine since ancient times. Synthetic work in this field[3] dates back to the 1940's. By Ruzicka, Plattner and their colleagues as well as Elderfield and his coworkers a large number of model experiments were carried out, and the methods were developed for the synthesis of the butenolide ring[4–6] and the introduction of 14β-hydroxy group[7,8] into model steroids. But the construction of both groups in the same molecule had not been achieved until 1962, when Sondheimer and coworkers announced an elegant synthesis of digitoxigenin.[9] Since their work there have been reported new routes to digitoxigenin[10] as well as successful syntheses of other naturally occurring cardenolides: periplogenin,[11] uzarigenin,[12] and canargenin.[13]

All of the recorded syntheses, however, suffer from inherent disadvantage in that they start with rare steroids. With the objective of making cardenolides more accessible we decided

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* Dedicated to the memory of Prof. Eiji Ochiai.

[1] Location: *Gofuku, Toyama.*

[2] For a review, see L.F. Fieser and M. Fieser, "Steroids," Chapter 20, Reinhold Publishing Corp., New York, 1959.

[3] For recent reviews, see F. Sondheimer, *Chemistry in Britain*, **1965**, 454; R. Deghenghi, *Pure and Applied Chemistry*, **21**, 153 (1970); R. Thomas, J. Boutagy, and A. Gelbart, *J. Pharm. Sci.*, **63**, 1649 (1974).

[4] L. Ruzicka, T. Reichstein, and A. Fuerst, *Helv. Chim. Acta*, **24**, 76 (1941); L. Ruzicka, P.A. Plattner, *et al.*, *ibid.*, **24**, 716 (1941); *idem*, *ibid.*, **25**, 65, 79 (1942); *idem*, *ibid.*, **26**, 2274 (1943); *idem*, *ibid.*, **27**, 988 (1944); *idem*, *ibid.*, **28**, 173 (1945); *idem*, *ibid.*, **29**, 248 (1946).

[5] R.G. Linville, J. Fried, and R.C. Elderfield, *J. Org. Chem.*, **7**, 362 (1942).

[6] L. Ruzicka, P.A. Plattner, and J. Pataki, *Helv. Chim. Acta*, **25**, 425 (1942).

[7] P.A. Plattner, L. Ruzicka, H. Heusser, J. Pataki, and K. Meier, *Helv. Chim. Acta.*, **29**, 942 (1946); L. Ruzicka, P.A. Plattner, H. Heusser, and K. Meier, *ibid.*, **30**, 1342 (1947); P.A. Plattner, L. Ruzicka, H. Heusser, and E. Anliker, *ibid.*, **30**, 385, 395 (1947).

[8] F. Sondheimer, S. Burstein, and R. Mechoulam, *J. Am. Chem. Soc.*, **82**, 3209 (1960).

[9] N. Danieli, Y. Mazur, and F. Sondheimer, *J. Am. Chem. Soc.*, **84**, 875 (1962); *Tetrahedron*, **22**, 3189 (1966).

[10] W. Fritsch, U. Stache, W. Haede, K. Radscheit, and H. Ruschig, *Ann.*, **721**, 168 (1969).

[11] R. Deghenghi, A. Phillip, and R. Gaudry, *Tetrahedron Letters*, **1963**, 2045.

[12] U. Stache, W. Fritsch, W. Haede, and K. Radscheit, *Ann.*, **726**, 136 (1969).

[13] W. Fritsch, H. Kohl, U. Stache, W. Haede, K. Rudscheit, and H. Ruschig, *Ann.*, **727**, 110 (1969).

to develop practical and general synthetic methods starting with ordinary and readily available steroids, *e.g.* pregnenolones or pregnanolones. What we had undertaken was in the first place to establish efficient transformation of these into pregn-14-en-20-ones which bear potential double bond for 14β-hydroxy group introduction, secondly the exploration of new butenolide synthesis applicable to pregn-14-en-20-ones. Successful results obtained are described below in separate sections.

Synthesis of Pregn-14-en-20-ones[14]

As indicated in the existing methods for the synthesis of natural cardenolides, it is essential to secure the 14-double bond in a steroid molecule for obtaining 14β-hydroxy group. For instance, digitoxigenin synthesis by German chemists[10] started with 15α-hydroxycortexone (microbiological oxidation product of desoxycorticosterone)[15] which on some stage of the synthesis was dehydrated to 14-double bond. The same authors also reported the preparation of pregna-5,14-dien-3β-ol-20-one[16] as an alternative candidate, from pregna-5,14-16-trien 3β-ol-20-one by a four step sequence that involved sodium-propanol reduction of the conjugated enone system to 14-en-20-ol. Although the latter synthetic method of pregn-14-en-20-one was attractive with respect to availability of the starting material,[17] the yield of the reduction was revealed to be low. If the selective reduction of the 16-double bond of 14,16-dien-20-ones could be accomplished in good yields and in single operation, the first problem in the present investigation will become solved. With this object in mind, pregnatrienolone acetate (**2**) was prepared from pregnadienolone acetate (**1**) and the model substrate was allowed to react with various reducing reagents capable of conjugate addition. The satisfactory results were obtained with triphenylstannane and some trisubstituted silanes as described below.

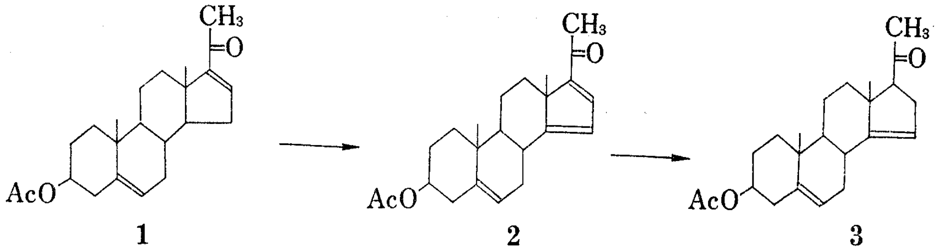

Chart 1

Reduction of 2 with Stannanes

The representative monohydrides, triphenylstannane and tri-*n*-butylstannane, were employed.[18] Triphenylstannane proved to be far more reactive. By heating the reagent with **2** in an inert solvent, pregn-14-en-20-one (**3**) was obtained in more than 80% yield. Only side reaction observed was the overreduction of 20-keto group to the corresponding alcohol which was easily suppressed by adjusting the addition of the reagent. On the other hand,

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[14] For previous communication on this subject and synthesis of digitanols thereof, see E. Yoshii and M. Yamasaki, *Chem. Pharm. Bull.* (Tokyo), **16**, 1158 (1968); E. Yoshii, H. Ikeshima, and K. Ozaki, *ibid.*, **20**, 1827 (1972).

[15] M. Shirasaka and M. Tsuruta, *Chem. Pharm. Bull.* (Tokyo), **9**, 238 (1961); S.H. Eppstein, P.D. Meister, D.H. Peterson, H.C. Murrag, H.M.L. Osborn, and A. Weintraub, *J. Am. Chem. Soc.*, **80**, 3382 (1958).

[16] H. Heusser, M. Roth, O. Rohr, and R. Anliker, *Helv. Chim. Acta*, **38**, 1178 (1955).

[17] A.J. Solo and B. Singh, *J. Org. Chem.*, **30**, 1658 (1965); R. Tschesch, F. Riemhofer, and G. Snatzke, *Chem. Ber.*, **98**, 1188 (1965).

[18] Application of these reagents for the hydrogenation of α,β-unsaturated ketones, see M. Pereyre and J. Valade, *Bull. Soc. Chim. France*, **1967**, 1928; D.R.G. Brimage, R.S. Davidson, and P.F. Lambeth, *J. Chem. Soc.*, (*C*), **1971**, 1241.

the reaction of tri-*n*-butylstannane with **2** proceeded sluggishly even in the presence of azobisisobutyronitrile giving maximum yield of 36%.

Since our preliminary report of this elegant and potentially useful method for the partial reduction of conjugated dienone,[14] several successful applications have been reported for other pregna-14,16-dien-20-ones[19] as well as terpenoid dienones and trienones.[20]

Reduction of 2 with Silanes

Initially we adopted a hydrosilation method of Petrov[21] who developed the conjugate addition reaction of triethylsilane across α,β-unsaturated ketones or aldehydes by chloroplatinic acid catalysis. The addition of triethylsilane upon our model compound **2** did occur in the presence of Speier's catalyst (chloroplatinic acid in isopropanol) by heating up to 145° for 5 hr, and pregn-14-en-20-one (**3**) was isolated in *ca.* 80% yield after acetic acid catalyzed hydrolysis of the enol silyl ether followed by silica gel chromatography. However, above silane reduction required a sealed tube reaction owing to low boiling point of the reagent and rather long reaction time, and hence we were directed to finding out more reactive silanes.

Four groups of trisubstituted silanes (alkyl, chloro, alkoxy, and siloxy) were subjected to the reaction with **2** giving the result summarized in Table I. Trialkylsilanes were least reactive, though the isolated yields were good. Other silanes were far more reactive, the hydrosilation step being completed at room temperature or by gentle warming. Particularly, ethoxydiethylsilane and tetramethyldisiloxane were found to be desirable reagents from the standpoints of the yields and of freedom from any appreciable side reactions. On the other hand, chlorosilanes and diethoxysilane gave lower yields, accompanied by fair amount of overreduction products. This side reaction which could not be suppressed may arise from that enol silyl ether intermediate readily underwent an acid catalyzed alcoholysis by the catalyst solution giving free 20-ketone which was then rapidly reduced.

TABLE I. The Reduction of Pregna-5,14,16-trien-3β-ol-20-one acetate (**2**) with Silanes

Silanes	Temp. (°C)	Reaction time[a] (hr)	% Yield of **3**[b]
Et_3SiH	150[c]	5	80
n-Pr_3SiH	160[c]	7	*ca.* 70[d]
$MeCl_2SiH$	60	0.5	*ca.* 50[e]
Me_2ClSiH	60	0.5	45[d]
$Et_2(EtO)SiH$	60	0.5	75—80
$Me(EtO)_2SiH$	room temp.	0.4	40[e]
$(Me_2SiH)_2O$	room temp.	0.5	75—80
$(MeHSiO)_4$	60	*f*)	*ca.* 50

a) Time required for disappearance of the starting material **2**.
b) Yields indicated are those obtained from the reactions employing 100 mg of **2**, 0.3—0.5 ml of toluene, and 0.04 ml of Speier's catalyst. **3** was isolated by silica gel chromatography after the reaction mixture was heated with acetic acid.
c) Reaction run in a sealed tube.
d) Overreduction product (pregna-5,14-dien-3β,20-diol 3-acetate) was obtained as by-product.
e) An unidentified overreduction product was also obtained.
f) Reaction interrupted by the formation of viscous polysiloxane.

Synthesis of Cardenolides

Having been accomplished the primary objective to establish the facile preparative method of pregn-14-en-20-ones, we directed our effort to exploring a practical synthesis of

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19) T. Nambara, K. Shimada, and S. Goya, *Chem. Pharm. Bull.* (Tokyo), **18**, 453 (1970); U. Pommerenk, H. Sengewein, and P. Welzel, *Tetrahedron Letters*, **1972**, 3415.
20) M. Yamasaki, *Chem. Comm.*, **1972**, 606; H.R. Wolf and M.P. Zink, *Helv. Chim. Acta.*, **56**, 1062 (1973).
21) A.D. Petrov and S.I. Sadykh-Zade, *Doklady Akad. Nauk SSSR*, **121**, 119 (1958); *Chem. Abstr.*, **53**, 1207 (1959).

cardenolides from them. The pressing requirement here is to obtain an effective synthetic method of butenolide ring from the 17β-acetyl side chain. A fair number of butenolide syntheses from methyl ketones have been recorded since the early 1940's but those applicable in the present case seemed to be very few.[3,22] Any reactions to affect the reactive 14-double bond should be avoided or strictly controlled. We describe below two different successful approaches exemplified by the syntheses of xysmalogenin and digitoxigenin.

Xysmalogenin[3β,14β-Dihydroxycarda-5,20(22)-dienolide, 9][23]

This naturally occurring cardenolide[24] was synthesized from pregna-5,14-dien-3β-ol-20-one acetate (**3**) by practically four step sequence which involved a modification of the existing methods.

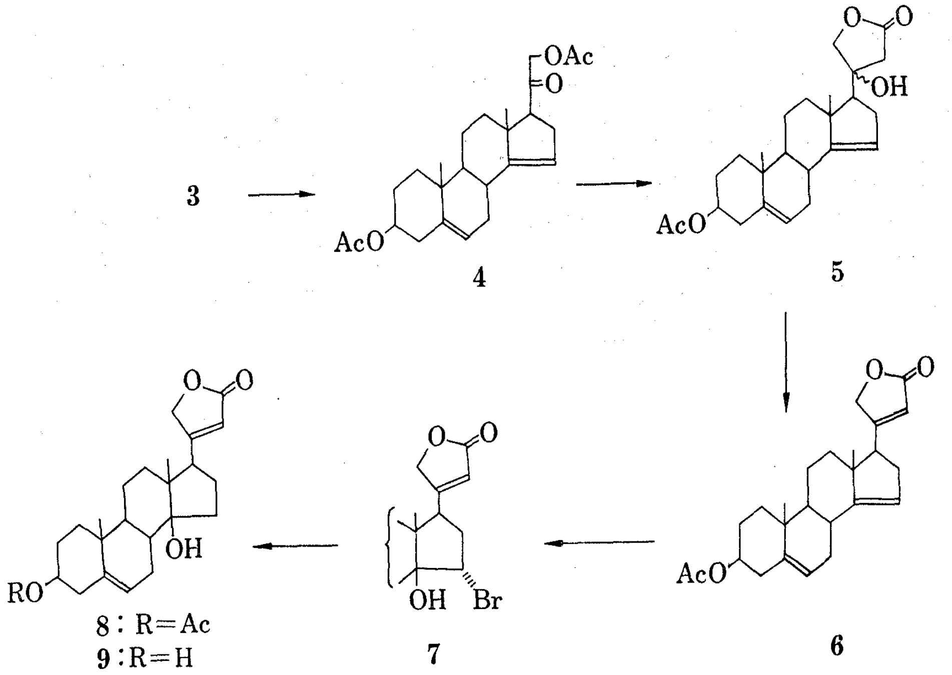

Chart 2

Introduction of 21-acetoxy group to **3** was most satisfactorily achieved by lead tetraacetate oxidation under boron trifluoride catalysis (Henbest method)[25] and by controlling the reaction temperature at 5—10°, providing 53% yield of **4**. Alternative traditional method involving 21-iodination through oxalyl derivative[10] or 20(21)-en-20-ol acetate[26] followed by displacement with potassium acetate resulted in inferior overall yield (below 30%). The second step, building up the 17β-butenolide ring was best achieved[27] by Reformatsky reaction with ethyl bromoacetate and zinc, the method utilized by Ruzicka, *et al.* in their synthesis of D-ring saturated cardenolides from 21-acetoxypregnan-20-ones. For obtaining maximum yield the reaction time should be as short as possible to exclude further ester condensation. Thus, by performing the reaction in boiling benzene for less than 20 min there was obtained in high yields β-hydroxy-γ-lactone (**5**) which by NMR proved to be a mixture of diastereomers (ratio,

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22) Y.S. Rao, *Chem. Rev.*, **64**, 360 (1964); H. Kroeper, "Methoden der Organischen Chemie (Houben-Weyl)", Vol. VI/2, p-571, Georg Thieme Verlag, Stuttgart, 1963.

23) For preliminary account, see E. Yoshii and K. Ozaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 1585 (1972).

24) J. Polonia, H. Kurtitzkes, H. Jager, and T. Reichstein, *Helv. Chim. Acta*, **46**, 8 (1963); R. Tschesche, W. Freytag, and G. Snatzke, *Chem. Ber.*, **92**, 3053 (1959).

25) J.D. Cocker, H.B. Henbest, G.H. Phillipps, G.P. Slater, and D.A. Thomas, *J. Chem. Soc.*, **1965**, 6.

26) C. Djerassi and C.T. Lenk, *J. Am. Chem. Soc.*, **76**, 1772 (1954).

27) For other inferior methods, see Reference 23), footnote 11).

ca. 1: 3). The major isomer presumably of 20β-hydroxy configuration was, to our gratefulness, readily dehydrated on alumina column chromatography to give anhydro-xysmalogenin acetate (**6**, 60% yield from **4**), whereas minor isomer was eluted unchanged from the column (13.5% yield from **4**) and could be dehydrated by means of boiling acetic anhydride.

The last stage of xysmalogenin synthesis that is the introduction of 14β-hydroxy group was performed by the well established method.[12-14] Anhydro-xysmalogenin acetate was treated with 1.2 equivalents of N-bromoacetamide in aqueous acetone to produce 15α,14β-bromohydrin (**7**) which was immediately subjected to hydrogenolysis with Raney Ni. Chromatography of the crude product on silica gel afforded 59% yield of xysmalogenin acetate (**8**). Finally, the 3-acetate group was hydrolyzed with 1N hydrochloric acid in methanol at room temperature yielding xysmalogenin (**9**), which was identified with natural specimen.[28]

Digitoxigenin[3β,14β-Dihydroxycard-20(22)-enolide, 24]

Although the foregoing synthesis of xysmalogenin demonstrated that in cardenolide synthesis pregn-14-en-20-ones are the useful key intermediates, there existed a relatively low yield step—21-acetoxylation of pregna-5,14-dien-20-one (**3**). This is not unexpected, since lead tetraacetate attacks olefinic bond too. Therefore, we decided to devise more efficient and broadly applicable synthetic method of cardenolides. The new method that we established consists of essentially three stages: (1) 21-methylsulfenylation of pregn-14-en-20-one,[29] (2) the resulting methylthiomethyl ketone is subjected to Reformatsky condensation with methyl bromoacetate and zinc, (3) α,β-unsaturated γ-lactone formation by base treatment of the S-methylated Reformatsky product.

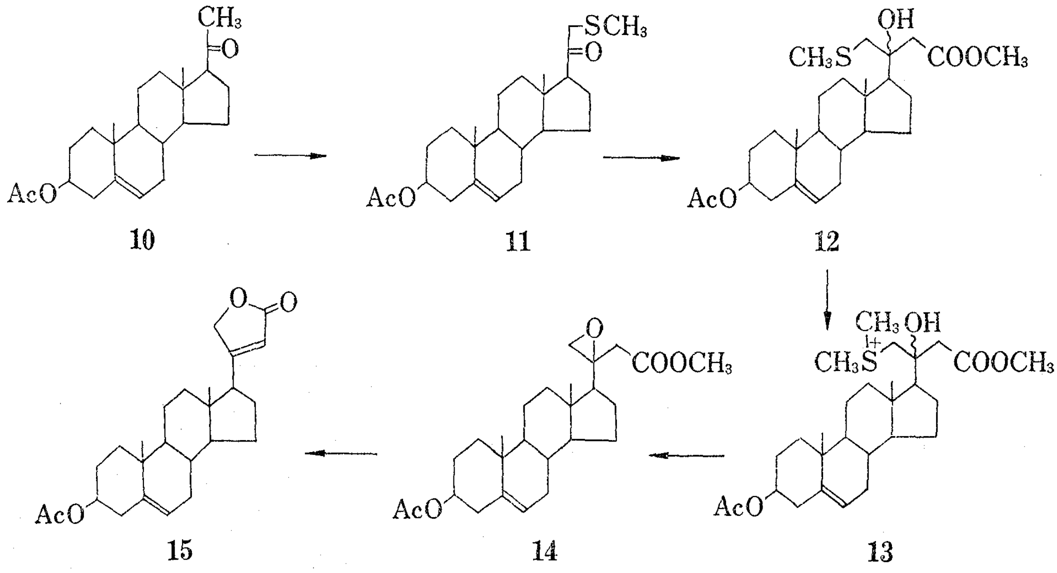

Chart 3

First, a model experiment of this overall process carried out with pregn-5-en-3β-ol-20-one acetate (**10**) is described. The pregnenolone acetate was condensed with diethyl oxalate in the presence of sodium methoxide, and the resulting crude oxalyl ketone was reacted with a slight excess of methyl thiotosylate in refluxing ethanol containing excess potassium acetate to give 83% yield of 21-methylthio derivative (**11**).[30] The reaction of **11** with methyl bromoacetate and zinc dust in boiling benzene proceeded almost instantaneously giving 85% yield

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[28] Natural xysmalogenin was kindly provided by professor Reichstein.

[29] E. Yoshii, T. Miwa, T. Koizumi, and E. Kitatsuji, *Chem. Pharm. Bull.* (Tokyo), 23, 462 (1975).

[30] Methylsulfenylation of cyclohexanones *via* 2-hydroxymethylene derivative: R.L. Autrey and P.W. Scullard, *J. Am. Chem. Soc.*, **90**, 4917, 4924 (1968); Y. Shimizu, *Tetrahedron Letters*, **1972**, 2919.

of 20-hydroxy-21-methylthio-24-nor-cholenoate (**12**, mixture of diastereomers). This Reformatsky product was then methylated with either trimethyloxonium tetrafluoroborate or methyl iodide to give the corresponding methylsulfonium salts (**13**) in quantitative yields. Finally, **13** was brought in the reaction with bases, with the expectation that it should eliminate dimethylsulfide to give epoxy ester (**14**) which subsequently might undergo *trans* β-eliminative epoxide cleavage leading to butenolide ring. By treatment of **13** with 0.15 N methanolic sodium methoxide at room temperature the expected reaction occurred in fact giving 3β-hydroxycarda-5,20(22)-dienolide in 53% yield, but accompanied with two by-products: methyl (*E*)-3β,21-dihydroxy-24-nor-chola-5,20(22)-dienoate (15%) and 3β-hydroxy-21-methoxycard-5-enolide (22%). The result indicated that the yield of the butenolide could be improved, if (1) the reaction condition were kept mild to prevent the formation of the latter by-product (secondary reaction product of the butenolide) and (2) upon base catalyzed epoxide cleavage such conformation of the side chain as *syn* arrangement of ester group and epoxide methylene were retained.[31] After several unsuccessful attempts along this line, we finally could find a simple procedure obtaining high yields in the ring closure.

By vigorous stirring of dichloromethane solution of β-hydroxysulfonium salt (**13**) with dilute aqueous sodium hydroxide, there was obtained in nearly quantitative yield the corresponding epoxy ester (**14**) as a mixture of 20-stereoisomers, separable by silica gel thin-layer chromatography. Column chromatography of the mixture using basic alumina did afford cardenolide (**15**) in 92% yield. The exclusive formation of the butenolide ring could be reasoned as such that by a steric factor the side chain of **14** was adsorbed on alumina taking required conformation as discussed above. Preparatively, this new synthetic method of cardenolide from **12** could be carried out without the isolation of intermediates in high overall yield.

With a new effective method for cardenolide synthesis at hand, we subsequently attempted the synthesis of digitoxigenin. Starting with commercially available pregn-16-en-3β-ol-20-one acetate or pregnan-3β-ol-20-one acetate (**16**), pregna-14,16-dien-20-one (**18**) was prepared by the following methods. Allylic bromination of the former with N-bromosuccinimide or bromine followed by dehydrobromination yielded the dienone (**18**) in 50—60% conversion yields, but great difficulty was encountered in isolating the product from unreacted starting material showing no preparative value. Also, 21-bromination with cupric bromide after the method of Glazier[32] followed by lithium halide catalyzed dehydrobromination did not afford fruitful result (less than 30% overall yield). The low yield was proved to be responsible to the formation of 21,21-dibromopregnenolone that could not be suppressed. Eventually, we established a high yield preparative method of **18** which involved 17,21-dibromination of **16** and subsequent dehydrobromination in the presence of lithium bromide.[33]

The dienone **18** was then converted to the enone **19** in good yields by reduction with triphenylstannane or triethylsilane. 21-Methylsulfenylation of **19** and subsequent Reformatsky reaction of the resulting 21-methylthiomethyl ketone (**20**) was done by the same procedure as above model experiment in the yields of 66 and 85%, respectively. Finally, 20-hydroxy-21-methylthio-24-nor-cholenoate (**21**) was S-methylated with trimethyloxonium tetrafluoroborate in nitromethane and the product was treated in dichloromethane with 0.15 N aqueous sodium hydroxide to give an epoxy ester (**22**), which on alumina chromatography was lactonized providing β-anhydrodigitoxigenin acetate (**23**)[34] in an overall yield of 85%. Since the trans-

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[31] W.W. Epstein and A.C. Sonntag, *J. Org. Chem.*, 32, 3390 (1967).
[32] E.R. Glazier, *J. Org. Chem.*, 26, 4397 (1962).
[33] R. Joly and J. Warnant, Fr. Patent addn., 71333 (1959) [*Chem. Abstr.*, **55**, 20008 (1961)]; R. Deghenghi, *Pure and Applied Chemistry*, **21**, 153 (1970).
[34] P.St. Janiak, E.K. Weiss, and T. Reichstein, *Helv. Chim. Acta*, **50**, 1249 (1967).

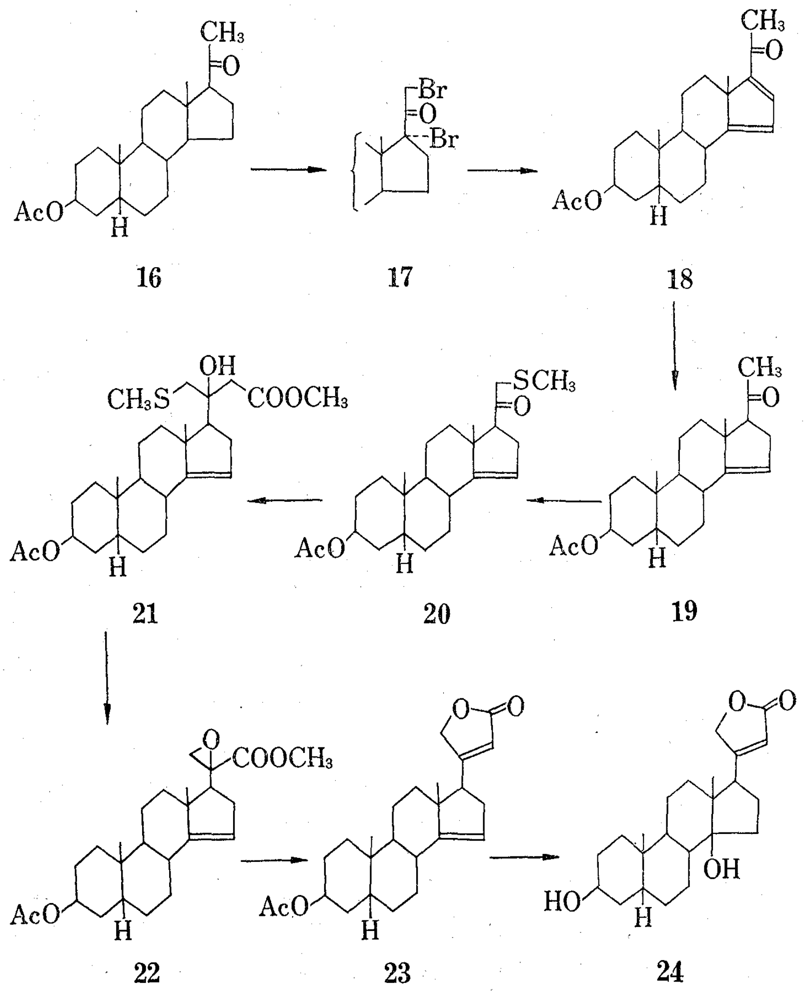

Chart 4

formation of **23** into digitoxigenin (**24**) *via* 15α,14β-bromohydrin has been established by several workers,[35] a new route to digitoxigenin was thus completed.

In conclusion, we could have established a facile preparative method of pregn-14-en-20-ones, which are key intermediates in the synthesis of cardenolides as well as related steroids such as bufadienolides[29] and digitanols.[14] Furthermore, our new and efficient cardenolide synthesis as illustrated in digitoxigenin synthesis appears to be quite general and should serve for the synthesis of other natural and synthetic cardenolides.

Experimental

Melting points were determined with Yanagimoto micro melting point apparatus and uncorrected. Infrared spectra (IR) were recorded on Nippon Bunko IR–S spectrometer and were calibrated using 1603 cm^{-1} polystyrene band. The 60-MHz nuclear magnetic resonance (NMR) spectra were taken with JEOL H-60 or PMX-60 spectrometer using TMS as internal standard in deuteriochloroform or carbon tetrachloride. Chemical shifts are reported in ppm (δ) from TMS. Mass spectra were obtained with JEOL JMS-OlSG-2 instrument at 75 eV ionization potential. Thin-layer chromatography (TLC) was conducted using Merck sliica gel G and preparative TLC using 20×20 cm glass plate coated with 20 g of Merck silica gel $PF_{254+366}$. For column chromatography Merck silica gel with 0.06—0.20 mm particles or Merck alumina was used.

Pregna-14,16-dien-20-ones——3β-Acetoxypregna-5,14,16-trien-20-one (2) was prepared according to the published procedure[17] by allylic bromination of 3β-acetoxypregna-5,16-dien-20-one (1). The crude product was contaminated with varying amount of unreacted 1. It was purified by chromatography on silica gel

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[35] Ch.R. Engel and G. Bach, *Steroids*, 3, 593 (1964); P. Hofer, H. Linde, and K. Meyer, *Helv. Chim. Acta*, 45, 1041 (1962); References, 12—14).

eluting with benzene and benzene–ether mixtures. Crystallization from methanol afforded pale yellow needles of mp 162—163°. IR (KBr) cm^{-1}: 1730, 1643, 1520. NMR ($CDCl_3$) δ: 1.15, 1.20 (18- and 19-CH_3), 2.03 (3-OAc), 2.32 (21-CH_3), 5.45 (6-H), 6.02 (15-H, diffused t), 7.23 (16-H, d, $J=2$).

3β-Acetoxy-5β-pregna-14,16-dien-20-one (**19**) was prepared from 3β-acetoxy-5β-pregnan-20-one (**16**, commercial or obtained by catalytic hydrogenation of commercial 3β-acetoxy-5β-pregn-16-en-20-one). To a solution of 40 g of **16** in 400 ml of acetic acid was added during 25 min and at room temperature 36 g of Br_2 in 150 ml of acetic acid. After the consumption of Br_2 the mixture was poured into cold water. The white precipitate was filtered and crystallized from acetone to give 49.5 g of 17,21-dibromopregnanone (**17**),[36] mp 182—184° (contained very small amount of tribromo derivative). The filtrate was proved by NMR to contain about 50% of **17** which could be isolated by silica gel chromatography, along with 17α- and 21-bromopregnanones. A mixture of 5.18 g of the dibromoketone (**17**), 1.79 g of anhydrous lithium bromide and 40 ml of dry dimethylformamide was heated at 90—95° for 3 hr with stirring under argon. The resulting dark solution was diluted with 250 ml of water and thoroughly extracted with ether. The combined ether extract was washed with saturated $NaHCO_3$ and saturated NaCl, and dried over $MgSO_4$. The solvent was then evaporated and the residue was crystallized from *n*-hexane using active charcoal for decoloration to give 2.67 g of 3β-acetoxy-5β-pregna-14,16-dien-20-one (**18**), mp 112—113°. IR (KBr) cm^{-1}: 1740, 1635, 1522. NMR (CCl_4) δ: 1.09, 1.11 (18- and 19-CH_3), 1.98 (3-OAc), 2.23 (21-CH_3), 5.87 (15-H, diffused t), 7.08 (16-H, d, J=2.25). *Anal.* Calcd. for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05. Found: C, 77.59; H, 9.18. Additional yield (up to 15%) could be obtained by silica gel chromatography of the mother liquor.

Preparation of Pregn-14-en-20-ones by Reduction of Pregn-14,16-dien-20-ones——General procedures which were established for the model compound- 3β-acetoxypregna-5,14,16-trien-20-one (**2**) are described.

With Triphenylstannane: In a sealed tube was placed a solution of 1.40 g of **2** and 4.0 g of freshly distilled triphenylstannane in 2.5 ml of toluene, and it was heated at 140° for 4 hr. The reaction mixture was cooled, and the precipitate (hexaphenylditin) was removed by filtration and washed with *n*-hexane. The filtrate was evaporated under reduced pressure and the residue was chromatographed on 30 g of silica gel. After removal of further amount of hexaphenylditin by eluting with benzene, the column was eluted with benzene-ether mixtures to give 1.12 g of 3β-acetoxypregna-5,14-dien-20-one (**3**) and 0.24 g of 3β-acetoxy-20-hydroxypregna-5,14-diene, mp 176—178° after recrystallization from methanol. Analytical sample of **3** was obtained by recrystallization from isopropyl ether-benzene, mp 160—161°. *Anal.* Calcd. for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05. Found: C, 77.49; H, 9.09. For large scale preparations, the reduction was conveniently carried out at atmospheric pressure by utilizing either toluene or xylene as solvent and refluxing in the presence of catalytic amount of azobisisobutyronitrile.[19,20] The reaction was followed by TLC and generally completed within 5 hr, providing comparable yield with the sealed tube reaction.

With Triethylsilane: In a glass tube 600 mg of **2** was dissolved in a mixture of 2.2 ml of toluene and 1.8 ml of triethylsilane by gentle warming. Then, 0.15 ml of 0.2 M chloroplatinic acid solution in isopropanol was added to the solution, and the tube was sealed and heated at 145° for 5 hr. After opening the tube 0.8 ml of acetic acid was added and the mixture was heated in boiling water bath for 1 hr. It was diluted with benzene, washed with saturated $NaHCO_3$, dried on $MgSO_4$, and evaporated. The residue was chromatographed on 20 g of silica gel eluting with benzene and benzene–ether mixtures. There were obtained 482 mg of crystalline fractions which showed single spot on TLC. The product **3** is pure enough for further reactions.

With Tetramethyldisiloxane: To a solution of 100 mg of **2** in 0.5 ml of toluene were added 0.08 ml of the silane and 0.04 ml of 0.2 M chloroplatinic acid solution of isopropanol. The mixture was allowed to stand at room temperature for about 30 min (if unreacted **2** was detected on TLC, additional catalyst and silane may be added). After the addition of few drops of acetic acid it was heated on a boiling water bath for 1—2 hr and then processed as described above. Silica gel chromatography of the crude product gave crystalline fractions totalling 75—80 mg.

Spectral data of two pregn-14-en-20-ones appeared in this paper are given below. **3**, IR (KBr) cm^{-1}: 1738, 1710; NMR ($CDCl_3$) δ: 0.87 (18-CH_3), 1.03 (19-CH_3), 2.03 (3-OAc), 2.15 (21-CH_3), 5.13 (15-H, m), 5.38 (6-H, m). **19** (not crystallizable), IR (film) cm^{-1}: 1730, 1705, 1233; NMR (CCl_4) δ: 0.80 (18-CH_3), 0.96 (19-CH_3), 1.95 (3-OAc), 2.05 (21-CH_3), 4.95 (3α-H, m), 5.10 (15-H, m).

3β,21-Diacetoxypregna-5,14-dien-20-one (**4**)——To a stirred solution of 5.20 g of 3β-acetoxypregna-5,14-dien-20-one (**3**) in 200 ml of benzene were added at 5° 11 ml of dry methanol and 26 ml of 45% boron trifluoride etherate. After 20 min 7.6 g of lead tetraacetate was added to the mixture and stirring at 5—10° was continued for 4.5 hr. The solution was washed with saturated $NaHCO_3$, dried on $MgSO_4$, and evaporated. The residue was chromatographed on 40 g of silica gel to give 680 mg of unreacted **3** by elution with benzene–*n*-hexane and 2.8 g of acetoxylation product **4** by elution with benzene. The product was recrystallized from isopropyl ether–benzene to give leaflets, mp 192—195°. IR (KBr) cm^{-1}: 1745 (sh), 1725. NMR (CCl_4) δ: 0.88 (18-CH_3), 1.03 (19-CH_3), 1.96 (3-OAc), 2.12 (21-OAc), 4.32, 4.68 (21-CH_2, d, $J=16$), 5.10 (15-H), 5.35 (6-H). *Anal.* Calcd. for $C_{25}H_{34}O_5$: C, 72.43; H, 8.26. Found: C, 72.11; H, 8.02.

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36) R.E. Marker, H.M. Crooks, Jr., and R.B. Wagner, *J. Am. Chem. Soc.*, **64**, 213 (1942).

3β-Acetoxycarda-5,14,20(22)-trienolide (6, Anhydroxysmalogenin Acetate)——To a solution of 200 mg of acetoxy ketone **4** in 20 ml of benzene was added 4 g of activated granular zinc, and about 5 ml of benzene was removed by distillation. Then, 1 g of ethyl bromoacetate was added and the mixture was gently refluxed with stirring. After the reaction started as indicated by vigorous refluxing, heating and stirring were continued for 15 min. Dilute acetic acid was introduced to the cooled reaction mixture, and benzene layer was washed with water, saturated $NaHCO_3$, and water, and dried. Evaporation of the solvent left a pale yellow solid which was crystallized from isopropyl ether-$CHCl_3$ to give 190 mg of crude **5** as white needles, mp 216—22° (close two spots on TLC due to diastereoisomers). IR (KBr) cm^{-1}: 3500, 1780, 1730. NMR ($CDCl_3$) δ: major isomer, 1.07 (18- and 19-CH_3), 2.95 (22-CH_2, br. s), 4.44, 4.79 (21-CH_2, d, $J=10$); minor isomer, 1.03 (19-CH_3), 1.10 (18-CH_3), 2.65 (22-CH_2, s), 4.27 (21-CH_2, s).

This β-hydroxy-γ-lactone mixture (**5**) was chromatographed on neutral alumina (activity III, 10 g), eluting successively with benzene–*n*-hexane (1: 1), benzene, and benzene-ethyl acetate (19: 1) to give the following compounds. (1) recovered starting material, 25 mg (12.5%). (2) anhydro-xysmalogenin acetate (**6**), 114 mg (60%), mp 171—172°, colorless needles from isopropyl ether–benzene. IR (KBr) cm^{-1}: 1790, 1770, 1730, 1630. NMR ($CDCl_3$) δ: 0.85 (18-CH_3), 1.05 (19-CH_3), 2.05 (3-OAc), 4.80 (21-CH_2, diffused s), 5.30 (15-H), 5.45 (16-H), 5.95 (22-H, diffused s). (3) anhydro-xysmalogenin, 5 mg (3%), mp 225°. (4) β-hydroxy-γ-lactone (minor isomer of **5**), 27 mg (13.5%), mp 270—274° from MeOH. IR (KBr) cm^{-1}: 3400, 1785, 1730. *Anal.* Calcd. for $C_{25}H_{34}O_5$: C, 72.43; H, 8.26. Found: C, 72.39; H, 8.27. This compound was dehydrated to **6** by refluxing with acetic anhydride in the presence of potassium acetate.

3β,14β-Dihydroxycarda-5,20(22)-dienolide (9, Xysmalogenin)——To a solution of 150 mg of anhydroxysmalogenin acetate (**6**) in 6 ml of acetone were added 0.15 ml of acetic acid and 0.5 ml of water. After cooling in an ice bath followed by addition of 65 mg of N-bromoacetamide, the mixture was stirred for 1 hr in a dark place. It was then poured on to saturated NaCl containing $Na_2S_2O_3$ and extracted with dichloromethane. The extract was washed with saturated NaCl, dried on Na_2SO_4, and evaporated at room temperature by a rotary evaporator. Crude 15α-bromo-14β-hydrin (**7**) obtained was dissolved in a mixture of 10 ml of methanol and 10 ml of dichloromethane, and added to a methanol suspension of 5 g of Raney Ni (W-4) which was previously saturated with hydrogen at 2 atm and adjusted to pH=6.7 by an acetate buffer. The mixture was stirred at room temperature for 2.5 hr, and then Raney Ni was removed by filtration. The filtrate was concentrated under reduced pressure and partitioned between dichloromethane and water. The organic layer was dried on Na_2SO_4 and evaporated. The residue was chromatographed on 7 g of silica gel. Elution with benzene recovered 20 mg of unreacted starting material (**6**), and successive elution with ethyl acetate-benzene (1: 19) afforded 82 mg of xysmalogenin acetate (**8**), mp 264—268° from isopropyl ether–MeOH (lit., mp 250—255°,[37] 258—269°[38]). IR (KBr) cm^{-1}: 3440, 1775, 1735, 1620. NMR ($CDCl_3$) δ: 0.98 (18-CH_3), 1.02 (19-CH_3), 2.05 (3-OAc), 2.7—3.0 (17α-H), 4.4—5.0 (3α-H), 4.95 (21-H, m), 5.45 (6-H, m), 5.93 (22-H, diffused s). Mass Spectrum *m/e*: 415 ($M^+ + 1$), 354 (M^+-AcOH), 336 (354-H_2O), 321 (336-CH_3), 201 ($C_{15}H_{21}$). ORD (dioxan) $[\alpha]^{22}$ (nm): +88.7° (270). *Anal.* Calcd. for $C_{25}H_{34}O_5$: C, 72.46; H,8.21. Found: C, 72.28; H, 8.40.

Xysmalogenin (**9**) was obtained by hydrolysis of the acetate **8** with 1 N methanolic HCl at room temperature for 20 hr, followed by preparative TLC using solvent system of ethyl acetate–$CHCl_3$ (1 : 1), mp 238—243° after crystallization from ethyl acetate (lit., 230—248°,[37] 247—249°[39]). IR (KBr) cm^{-1}: 3500, 3400, 1785, 1735, 1615. Mass Spectrum *m/e*: 372 (M^+), 354 (M^+–H_2O), 321 (336–CH_3). The mass[40] and IR spectra of synthetic xysmalogenin was indistiguishable with those of natural sample which was provided by professor Reichstein.

3β-Acetoxy-21-methylthiopregn-5-en-20-one (11)——To a suspension of 5.95 g of sodium methoxide in 300 ml of benzene was added 10.22 g of diethyl oxalate and 12.5 g of 3β-acetoxypregn-5-en-20-one and the mixture was stirred under nitrogen. After 18 hr 500 ml of 2 M NaH_2PO_4 and some ether were introduced, and the organic layer was separated, washed with water, dried on Na_2SO_4, and evaporated. To the residue dissolved in 300 ml of dry ethanol were added 17.2 g of anhydrous potassium acetate and 7.8 g of methyl thiotosylate, and the mixture was heated at 50° for 3.5 hr under nitrogen. After cooling to room temperature it was stirred with 60 ml of 10% KOH for 15 min, neutrallized with HCl, concentrated by a rotary evaporator, and extracted with ether. The ether solution was washed with water and saturated NaCl, dried on $MgSO_4$, and evaporated. The residue was crystallized from isopropyl ether to give 6.2 g of 3β-hydroxy-21-methylthiopregn-5-en-20-one as pale yellow crystals, mp 95—105°. Additional yield (3.4 g) was obtained from the mother liquor by silica gel chromatography. IR (KBr) cm^{-1}: 1695. NMR ($CDCl_3$) δ: 0.68 (18-CH_3), 1.02 (19-CH_3), 2.07 (SCH_3), 3.15 (21-CH_2), 5.35 (6-H). The acetate **11** was obtained by acetylation with acetic anhydride and pyridine, mp 141—142.5°, prisms from ethyl acetate-EtOH. IR (KBr) cm^{-1}: 1720, 1690.

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38) W. Schmidt, H.P. Uehlinger, Ch. Tamm, and T. Reichstein, *Helv. Chim. Acta*, 42, 72 (1959).

39) L. Meister, H. Kaufmann, W. Stocklin, and T. Reichstein, *Helv. Chim. Acta*, 53, 1659 (1970).

40) P. Reichstein, W. Stocklin, and T. Reichstein, *Helv. Chim. Acta*, 50, 2139 (1967).

NMR (CCl_4) δ: 0.65 (18-CH_3), 1.03 (19-CH_3), 1.97 (3-OAc), 2.01 (SCH_3), 3.00 (21-CH_2), 5.33 (6-H). *Anal.* Calcd. for $C_{24}H_{36}O_3S$: C, 71.24; H, 8.97. Found: C, 71.39; H, 9.05.

3β-Acetoxy-21-methylthio-5β-pregn-14-en-20-one (20)——This compound was prepared by the same procedure described above. From 2.7 g of 3β-acetoxy-5β-pregn-14-en-20-one (19) there was isolated 1.8 g of 3β-hydroxy-21-methylthio-5β-pregn-14-en-20-one after silica gel chromatography, mp 96—98° from isopropyl ether. IR (KBr) cm^{-1}: 1695. NMR (CCl_4) δ: 0.82 (18-CH_3), 0.95 (19-CH_3), 2.02 (SCH_3), 3.02 (21-CH_2), 5.08 (15-H). Acetate (20): mp 124—126° from isopropyl ether–*n*–hexane. NMR (CCl_4) δ: 0.83 (18-CH_3), 0.97 (19-CH_3), 1.94 (3-OAc), 2.00 (SCH_3), 3.00 (21-CH_2), 4.90 (3α-H), 5.07 (15-H). Mass Spectrum *m/e*: 404 (M^+). *Anal.* Calcd. for $C_{24}H_{36}O_3S$: C, 71.24; H, 8.97. Found: C, 71.02; H, 8.73.

Methyl 3β-Acetoxy-20-hydroxy-21-methylthio-24-nor-chol-5-enoate (12)——A solution of 2.43 g of methylthiomethyl ketone **11** in 70 ml of benzene was distilled to remove 35 ml of the solvent, and then were added 458 mg of activated zinc dust and 0.9 ml of methyl bromoacetate. With vigorous stirring of the mixture additional 20 ml of benzene was slowly distilled off, when vigorous reaction set in and all of zinc dust disappeared. After cooling to room temperature, the benzene solution was washed with dilute acetic acid, saturated $NaHCO_3$, and dried on $MgSO_4$. Evaporation of the solvent afforded a crystalline mass which was crystallized from EtOH to give 1.95g of **12**, mp 126—128°. Silica gel chromatography of the mother liquor yielded 110 mg of the starting material and 410 mg of **12**. IR (KBr) cm^{-1}: 1710. NMR ($CDCl_3$) δ: 0.83 (18-CH_3), 1.00 (19-CH_3), 1.92 (3-OAc), 2.02 (SCH_3), 3.60 ($COOCH_3$), 5.27 (5-H). Mass Spectrum *m/e*: 478 (M^+), 418 (M^+–AcOH). *Anal.* Calcd. for $C_{27}H_{42}O_5S$: C, 67.74; H, 8.84. Found: C, 67.64; H, 9.13.

Methylsulfonium iodide (**13** iodide) was prepared by heating at 60—80° of **12** with excess methyl iodide in nitromethane, mp 149—150° (decomp.) from EtOH. *Anal.* Calcd. for $C_{28}H_{45}O_5SI$: C, 54.19; H, 7.31. Found: C, 54.11; H, 7.19.

Methylsulfonium tetrafluoroborate (**13** tetrafluoroborate) was prepared in quantitative yield by reacting 479 mg of **12** with 148 mg of trimethyloxonium tetrafluoroborate in 4 ml of nitromethane at room temperature for 1 hr, mp 195° (decomp.) from EtOH–ether. *Anal.* Calcd. for $C_{28}H_{45}O_5SBF_4$: C, 57.93; H, 7.81. Found: C, 57.81; H, 7.86.

Methyl 3β-acetoxy-20-hydroxy-21-methylthio-24-nor-5β-chol-14-enoate (21)——By reacting 900 mg of **20** with 175 mg of zinc dust and 0.35 ml of methyl bromoacetate by the same procedure as described above, there were obtained after silica gel chromatography 156 mg of unreacted **20**, and 675 mg of **21** which was recrystallized from isopropyl ether–*n*–hexane or EtOH, mp 82—85°. IR (KBr) cm^{-1}: 1725, 1705. NMR (CCl_4) δ: 0.97, 1.10 (18- and 19-CH_3), 1.94 (3-OAc), 2.09 (SCH_3), 3.63 ($COOCH_3$), 4.87 (3α-H), 5.03 (15-H). Mass Spectrum *m/e*: 478 (M^+), 418 (M^+–AcOH). *Anal.* Calcd. for $C_{27}H_{42}O_5S$: C, 67.74; H, 8.84. Found: C, 67.60; H, 8.99.

Reaction of Methylsulfonium Salt (13) with Base——a) To a solution of 174 mg of tetrafluoroborate of **13** in 2 ml of dry MeOH was added 0.3 ml of 0.15 N methanolic NaOMe at room temperature. On standing slow precipitation of crystals was observed. After 2 hr the solution was neutralized with acetic acid and the precipitate was filtered to give 46 mg of 3β-hydroxycarda-5,20(22)-dienolide.[41] Mass Spectrum *m/e*: 356 (M^+). Half of the filtrate was subjected to preparative TLC ($CHCl_3$–MeOH, 20: 1) and separated into three products: lower band 13 mg; upper band, 17 mg; and center band (3-hydroxycardadienolide), 7 mg.

The lower band was crystallized from ethyl acetate, mp 225—227°, and identified as methyl (*E*)-3β,21-dihydroxy-24-nor-chola-5,20(22)-dienoate. IR (KBr) cm^{-1}: 1700, 1625. NMR ($CDCl_3$) δ: 0.63 (18-CH_3), 0.97 (19-CH_3), 3.62 ($COOCH_3$), 4.20 (21-CH_2, br. s), 5.25 (6-H, m), 6.07 (22-H). Mass Spectrum *m/e*: 388 (M^+), 370 (M^+–H_2O), 355 (370-CH_3). *Anal.* Calcd. for $C_{24}H_{36}O_4$: C, 74.19; H, 9.34. Found: C, 74.18; H, 9.29.

The upper band, not crystallizable, was assigned to 21-epimeric mixture of 3β-hydroxy-21-methoxycard-5-enolide by spectral data. NMR (CCl_4) δ: 0.72, 0.80 (18-CH_3), 1.02 (19-CH_3), 3.67 (OCH_3). Mass Spectrum *m/e*: 388 (M^+), 370 (M^+–H_2O), 355 (370-CH_3).

b) To a solution of 220 mg of **13** (tetrafluoroborate) in 10 ml of dichloromethane was added 0.5 N NaOH and two layer mixture was vigorously stirred for 1 hr at room temperature. After neutralization with acetic acid the dichloromethane layer was taken, washed with water, and dried on $MgSO_4$. Evaporation of the solvent gave 156 mg of **14** as crystalline mass which was recrystallized from isopropyl ether, mp 140—145° (leaflets), mixture of diastereomers showing two spot on TLC (AcOEt–cyclohexane, 2: 5). They were separated by preparative TLC. Higher *Rf*: mp 127—131°. IR (KBr) cm^{-1}: 1728. NMR (CCl_4) δ: 0.80 (18-CH_3), 1.03 (19-CH_3), 1.94 (3-OAc), 3.64 ($COOCH_3$), 5.25 (6-H). Mass Spectrum *m/e*: 430 (M^+), 370 (M^+–AcOH), 338 (370-MeOH). *Anal.* Calcd. for $C_{26}H_{38}O_5$: C, 72.54; H, 8.89. Found: C, 72.34; H, 8.91. Lower *Rf*: 141—143°. IR (KBr) cm^{-1}: 1739, 1718. NMR (CCl_4) δ: 0.71 (18-CH_3), 1.03 (19-CH_3), 1.94 (3-OAc), 3.63 ($COOCH_3$). Mass Spectrum *m/e*: 430 (M^+), 370 (M^+–AcOH), 338 (370-MeOH).

3β-Acetoxycarda-5,20(22)-dienolide (15)——A solution of 81 mg of **14** in small volume of benzene–*n*–hexane mixture (1: 1) was charged on 10 g of alumina (basic, activity II). After 1 hr the column was eluted with benzene and benzene–ether mixtures. Crystalline fractions (69 mg) which showed single spot on TLC

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41) Identified by comparison of IR, TLC and mass spectrum with authentic sample prepared by known method.[4] See also, W. Kreiser and M. Nazir, *Ann.*, **755**, 12 (1972) and references cited therein.

were collected and recrystallized from isopropyl ether–AcOEt to give **15**, mp 176—178°. Identification was made by comparison of spectral data and TLC with the specimen prepared by Reformatsky reaction of 3β, 21-diacetoxypregn-5-en-20-one.[4] NMR ($CDCl_3$) δ: 0.65 (18-CH_3), 1.03 (19-CH_3), 4.70 (21-CH_2), 5.37 (6-H), 5.78 (22-H).

3β-Acetoxy-5β-carda-14,20(22)-dienolide (23, β-Anhydrodigitoxigenin Acetate)——To a solution of 159 mg of **21** in 5 ml of nitromethane was added 55 mg of trimethyloxonium tetrafluoroborate at room temperature. After 30 min the solvent was evaporated, and the residue was dissolved in 15 ml of dichloromethane and vigorously stirred with 10 ml of 0.5 N NaOH for 1hr. The mixture was neutralized with acetic acid, and the organic layer was separated, washed with water, dried on $MgSO_4$, and evaporated to give 145 mg of crystalline mass. NMR (CCl_4) δ: 0.97, 1.03 (18- and 19-CH_3), 1.97 (3-OAc), 3.57 ($COOCH_3$). This epoxy ester (**22**) consisted of 20-epimers which were separable by preparative TLC. More polar isomer: mp 99—100°. *Anal.* Calcd. for $C_{26}H_{38}O_5$: C, 72.52; H, 8.89. Found: C, 72.53; H, 8.65. Less polar isomer, mp 80—83° (isopropyl ether). *Anal.* Calcd. for $C_{26}H_{38}O_5$: C, 72.52; H, 8.89: Found: 72.69; H, 8.84.

This crude epoxy ester was then subjected to alumina column chromatography (basic, activity II, 15 g) eluting with benzene and 1—3% ether in benzene to give 112 mg (85% from **21**) of β-anhydrodigitoxigenin acetate (**23**), mp 183—185.5° after recrystallization from isopropyl ether (lit.,[34] mp 182—184°), $[\alpha]_D^{18} = -16.4°$ ($c=0.66$, $CHCl_3$). The IR was identical with that reported.[34] NMR ($CDCl_3$) δ: 0.82 (18-CH_3), 0.98 (19-CH_3), 2.03 (3-OAc), 4.71 (21-CH_2, br. s), 5.02 (3α-H, br. s), 5.20 (15-H, br. s), 5.83 (22-H, br. s). Mass Spectrum *m/e*: 398 (M^+), 338 (M^+–AcOH). *Anal.* Calcd. for $C_{25}H_{34}O_4$: C, 75.34; H, 8.60. Found: C, 75.36; H, 8.76.

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