Modified Cardenolides II

Synthesis, NMR Spectra, and Biological Activity of C-19 Halides and Sulfonates

By MANFRED E. WOLFF and WINSTON HO

The synthesis of 19-halocardenolides by halide displacement of the corresponding 19-*p*-toluenesulfonate esters is described. The NMR spectra of the 19-substituted compounds indicate that the rotation of the 19- group is restricted and that one of the C-19 protons is shielded by the 5,6-double bond.

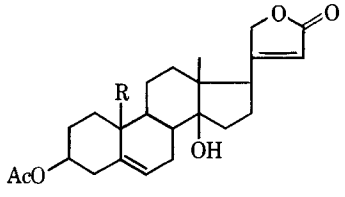
RECENT STUDIES (1) from this laboratory have allowed the formulation of a picture of steroid receptor interaction involving close spatial interaction of the 19-angular methyl group with a receptor surface. This concept was based on the effect on pharmacological activity of steroids with bulky C-19 substituents. It seemed useful to extend this sort of structure–function analysis to the cardiac aglycones.

It is known that the nature of the C-19 substituent in the aglycones has a profound effect on pharmacological activity (2). Thus, C-19 alcohols are more active than the corresponding aldehydes which are more active than the methyl compounds. These results have been obtained largely from naturally occurring glycosides; comparatively little has been done with respect to synthetic modification of the cardiac aglycones. Lingner *et al.* (3) prepared various derivatives of C-19 aldehydes and alcohols, including oximes, acetals, urethans, ethers, and esters. The nature of the substituent had a major effect on the oral activity of these compounds.

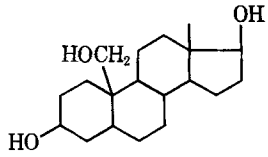
This paper describes the preparation, conformation, and structure–function relationships of C-19 halocardenolides.

DISCUSSION

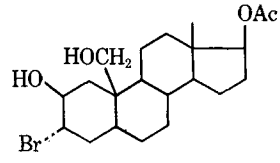
Chemistry—Work by others (4–8) has resulted in the production of C-19 halo-Δ^5-steroids from the corresponding 19-tosylate or mesylate esters. It has been established that homoallylic carbonium ion intermediates are involved, since S_N2 displacements on saturated 19-tosylates fail. Nucleophilic displacement of 19-tosylates by chloride or bromide gives 19-halides, but other nucleophiles, such as hydroxide, alkoxide, or azide give 5,19-cyclo-6-substituted steroids. It has been concluded that the latter are the kinetically favored products, but the



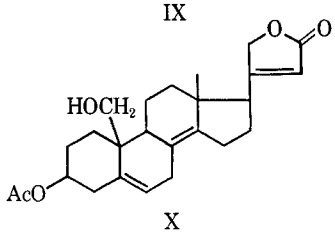
I, R = CHO
II, R = CH_2OH
III, R = $CH_2OSO_2{\cdot}C_6H_4CH_3$
IV, R = $CH_2OSO_2CH_3$
V, R = CH_2Cl
VI, R = CH_2Br
VII, R = CH_2I



VIII



IX



X

6-halides are unstable and rearrange further to afford the 19-halides.

The starting material for the present work was monoanhydrostrophanthidin acetate (I), formed from strophanthidin by elimination of the elements of water (9). This material was originally formulated as a 14-ene (10) but Fieser and Goto (11) assigned the corrected Δ^5 structure (I) on the basis of rotations and a reinterpretation of the nature of products of basic isomerization. The reactions of II described in this study, as well as the NMR spectrum of products derived from I, fully support the reassigned structure.

Received September 2, 1966, from the Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, CA 94122

Accepted for publication October 25, 1966.

This research was supported in part by grant HE-09578 from the National Heart Institute, U. S. Public Health Service, Bethesda, Md.

The authors wish to dedicate this paper to Dean T. C. Daniels, whose leadership at the University of California has done so much to raise the standards of education and research in the pharmaceutical sciences.

Previous paper: Wolff, M. E., Ho, W., and Katzung, B., *Chem. Ind. (London)*, **1965**, 1976.

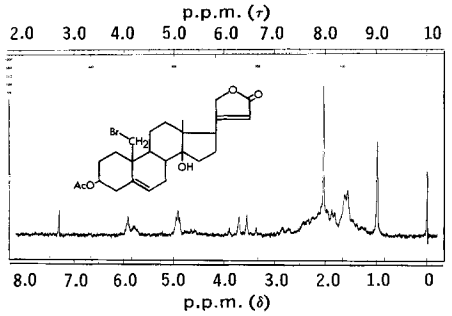


Fig. 1—NMR spectrum of 19-bromo-3β,14β-dihydroxycarda-5,20(22)-dienolide 3-acetate (VI).

Reduction of I with sodium borohydride in methanol solution gave the 19-alcohol (II). The tosylate (III) and the mesylate (IV) were prepared by treatment of II with the appropriate sulfonyl chloride in pyridine solution at 27°. The 19-halocardenolides (V, VI, and VII) were formed readily by the reaction of III and the appropriate lithium halide salt in boiling isopropanol solution.

NMR Spectra—An examination of the NMR spectra (Fig. 1) (Table I) of these C-19 modified steroids has revealed features which shed light on the conformation of the C-19 substituent under the conditions of measurement. Spectral features which did not vary include the 3β-acetate methyl resonance at 121–122 c.p.s., and the C-22 proton, seen centered at 353–354 c.p.s. as a singlet broadened by coupling to protons at C-21. The C-21 protons appear centered at 293–295 c.p.s. in a broadened triplet-like pattern owing to coupling to each other and to allylic coupling to the proton at C-22. The center of the "triplet" is actually the two central peaks of the AB system at C-21 and in a few spectra the satellite peaks can be discerned ($J_{AB} \cong 18$ c.p.s.). The side peaks in the "triplet" are actually due to allylic splitting (12) of the central peaks and $J_{21,22}$ is about 2 c.p.s., in agreement with an angle of about 90° between the plane of the double bond and each of the protons at C-21.

In the 19-halocardenolides (V, VI, and VII) as well as in the 19-hydroxy cardenolide (II), the two protons at C-19 produce an AB quartet having J_{AB} 11–13 c.p.s. which is typical of geminal coupling. Such a quartet could originate from restricted rotation of the 19-angular methyl group and also by selective shielding of one of the C-19 protons by the double bond. That restricted rotation alone does not account for the quartet is seen in the spectrum of the saturated compounds VIII (13) and IX (14), in which the C-19 protons produce broad singlets. The chemical shift of the C-19 protons in the simple saturated system (VIII) (235 c.p.s.), however, lies close to the computed position of one of the protons in II (231 c.p.s.). Moreover, chloride and hydroxyl exert deshielding effects of similar magnitude and it is noteworthy that in V also the low field peak is at 231 c.p.s. Thus, one of the peak positions is in the expected location for a proton of this type. The high field proton is at 215 and 214 c.p.s. in II and V, respectively. Molecular models of compounds II, V, VI, and VII show that the 19-substituent is most readily accommodated between the 2β- and 11β-hydrogens (Fig. 2), and thus the C-19 group is effectively locked into one position. Viewing the C-19 group from the top, this conformation places the lefthand hydrogen in a shielding environment due to the 5,6-double bond. The righthand hydrogen is more distant and appears to be in an area where neither shielding nor deshielding occurs. The spectrum of the doubly unsaturated compound X (15) was wholly in accord with this explanation. Models of X indicate that *both* C-19 hydrogens are located in a similar shielded environment and the spectrum indeed shows a single peak at 219 c.p.s. Thus, the difference in C-19 proton resonance positions between VIII and X is 16 c.p.s., the same value as found for $\delta_A - \delta_B$.

The tosylate (III) exhibits anomalous resonance from protons at C-18, C-6, and C-19. In all cases, the peaks occur at higher field than would be expected. That this shielding effect is due to the phenyl ring rather than the sulfonate group is shown by the spectrum of the mesylate (IV), in which no such anomalies occur.

Since the shielding due to the phenyl ring is seen in three widely separated points (C-6, C-18, and C-19), only the conformation shown in Fig. 3, in which the phenyl ring overlies the steroid, can account for the effect. From this it is also clear that it is the lefthand proton at C-19, rather than the righthand proton, which is shielded by the double bond at C-5, since in III, in which the righthand proton is clearly shielded, $\delta_A - \delta_B$ becomes *smaller*, rather than larger.

Biological Activity—The biological activity of the synthetic cardenolides was determined essentially by the method of Chen (16). Briefly, cats were anesthesized by intraperitoneal administration of 2 ml./Kg. of a freshly prepared aqueous solution containing in each ml. 30 mg. of α-chloralose and 250 mg. of urethan. The femoral vein was injected at 3-min. intervals with 0.5–1.0 ml. of 47.5% alcohol containing 0.2 mg. of drug. These doses

TABLE I—NMR SPECTRA OF C-19 SUBSTITUTED STEROIDS

Compd.	C-6-H($W_{1/2}$)	C-18-H	C-19-H δ_A	C-19-H δ_B	J_{AB}	$\delta_A - \delta_B$	C-21-H	C-22-H	C-3 Acetate
II	348 (10)	57	215	231	12	16	293 (m)	353	121
III	341 (10)	48	240	248	11	8	293 (m)	353	121
IV	348 (10)	57	251	267	11	16	293 (m)	354	121
V	347 (10)	58	214	231	13	17	294 (m)	354	121
VI	347 (10)	59	209	225	12	16	295 (m)	354	121
VII	344 (10)	60	196	215	12	19	294 (m)	354	122
VIII	...	47	235	($W_{1/2}$5)	..	..	...	...	...
IX	...	49	228	($W_{1/2}$3)	..	..	...	...	...
X	...	53	219	($W_{1/2}$4)	..	..	...	...	...

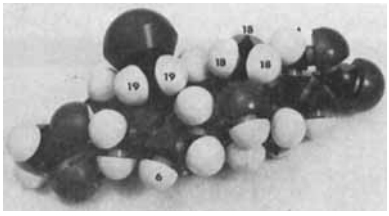


Fig. 2—Courtauld model of 19-iodo-3β,14β-dihydroxycarda-5,20(22)-dienolide 3-acetate (VII).

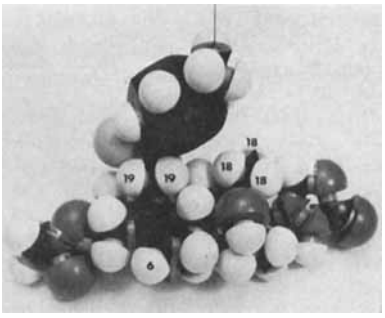


Fig. 3—Courtauld model of 3β,14β,19-trihydroxycarda-5,20(22)-dienolide 3-acetate 19-p-toluene sulfonate (III).

were designed to produce an end point at 30–60 min. Kg.[1] The effect of the drug was monitored by EKG readings.

Because of the lack of material, all compounds were assayed using only one animal. The lethal doses (mg./Kg.) are: III (1.6), V (0.4), and VI (1.5), but obviously no statistical analysis is possible. Arrhythmias typical of cardiotonic steroids were observed in the EKG in all cases. Although no quantitative evaluation can be made, it can nevertheless be concluded that these analogs are not inactive. Since both these compounds, with bulky β-face substituents, and A/B *cis* steroids, in which ring A is bent back, are active, it seems likely that the A ring is not in contact with the cellular receptor surface. This is supported by the activity of many other ring A and ring B modified cardenolide analogs which have been synthesized and tested in this laboratory (17), and is of particular interest in connection with the elegant work of Kupchan *et al.* (18). These workers found that strophanthidin 3-iodoacetate (SIA) irreversibly inhibits transport ATPase and concluded this was due to alkylation of the enzyme at the site where cardiotonic steroids bind. The present study, however, indicates that if transport ATPase is in fact the true receptor, the inhibition is due to exoalkylation (19) at a point removed from the active site. This is in harmony with the concept (20) of the steroid as a *haftgruppe* for the butenolide ring.

EXPERIMENTAL[2]

3β,14β,19-Trihydroxycarda-5,20(22)-dienolide 3-Acetate (II)—To a stirred suspension of 0.6 Gm. of I (9) in 50 ml. of tetrahydrofuran and 30 ml. of methanol, there was added 0.15 Gm. of sodium borohydride in 2 ml. of water. When the reaction was completed (1–2 hr., clear solution), 10 ml. of water was added and the solvent was evaporated under reduced pressure. More water was added, and the precipitated product was filtered, washed with water, and dried. There was obtained 0.55 Gm. of crude product, m.p. 245–252°. Recrystallization from acetonitrile twice gave 0.45 Gm. of crystals, m.p. 260–263°. Further recrystallization from the same solvent gave the analytical sample, m.p. 263–265°; $[\alpha]_D^{19}$ + 1° (c 1, $CHCl_3$).

Anal.—Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.34; H, 8.15.

3β,14β-19-Trihydroxycarda-5,20(22)-dienolide 3-Acetate 19-p-Toluenesulfonate (III)—A solution of 0.2 Gm. (0.00046 mole) of II and 0.2 Gm. of *p*-toluenesulfonyl chloride in 10 ml. of pyridine was kept at 27° for 48 hr. It was diluted with ice water and the resulting precipitate was collected and recrystallized from a mixture of methanol and a little acetonitrile to give 0.1 Gm. of colorless crystals, m.p. 155–157°. Further recrystallizations gave the analytical sample, m.p. 156–157°; $[\alpha]_D^{19}$ −41° (c 1, $CHCl_3$).

Anal.—Calcd. for $C_{32}H_{40}O_8S$: C, 65.77; H, 6.84. Found: C, 65.83; H, 6.52.

3β,14β-19-Trihydroxycarda-5,20(22)-dienolide 3-Acetate 19-Methanesulfonate (IV)—A solution of 0.4 Gm. (0.00092 mole) of II and 0.4 Gm. of methanesulfonyl chloride in 35 ml. of pyridine was kept at 27° for 48 hr. and worked up as described for III. Recrystallization from methanol gave the analytical sample, m.p. 145–146°; $[\alpha]_D^{20}$ −27° (c 1, $CHCl_3$).

Anal.—Calcd. for $C_{26}H_{36}O_8S$: C, 61.41; H, 7.14. Found: C, 61.36; H, 7.02.

19-Chloro-3β,14β-dihydroxycarda-5,20(22)-dienolide 3-Acetate (V)—A solution of 0.1 Gm. (0.00017 mole) of III and 0.1 Gm. (0.0023 mole) of lithium chloride in 12 ml. of isopropyl alcohol was heated under reflux for 3 hr. The solvent was evaporated under reduced pressure. The mixture was diluted with water and the resulting precipitate was collected and recrystallized three times from acetonitrile–methanol to give 0.05 Gm. of colorless crystals, m.p. 230–233°. Further recrystallizations gave the analytical sample, m.p. 242–244°; $[\alpha]_D^{19}$ −24° (c 0.6, $CHCl_3$).

Anal.—Calcd. for $C_{25}H_{33}ClO_5$: C, 66.87; H, 7.40. Found: C, 66.97; H, 7.36.

19-Bromo-3β,14β-dihydroxycarda-5,20(22)-dienolide 3-Acetate (VI)—A solution of 0.15 Gm. (0.00025 mole) of III and 0.15 Gm. (0.0017 mole) of lithium bromide in 15 ml. of isopropyl alcohol was heated under reflux for 2.5 hr. After evaporation of the solvent under reduced pressure, water was added to precipitate the product. Recrystallization from acetonitrile–methanol gave 0.06 Gm. of

[1] The authors are greatly indebted to Dr. Bertram Katzung and Mr. Charles Lee for help with the biological studies.

[2] Melting points were determined with a Thomas-Hoover apparatus equipped with a corrected thermometer. Infrared spectra were obtained with a Beckman IR-8 or Perkin Elmer 337 instrument. Microanalyses were performed by the Microanalytical Department, University of California, Berkeley, Calif. Optical rotations were obtained in a 0.5-cm. tube with a Rudolph photoelectric polarimeter. NMR spectra were obtained at a field strength of 60 Mc./sec. on samples in deuteriochloroform solution on a Varian A-60 or A-60A instrument, using tetramethylsilane as internal standard. Peak positions of AB quartets were computed with a punch card program on a Wyle calculator. When only small amounts of sample were available, or when the sample was poorly soluble in deuteriochloroform, a Varian C-1024 computer was used for time averaging.

product, m.p. 190–194°. The analytical sample had m.p. 195–197°; $[\alpha]_D^{19}$ −28° (c 1, $CHCl_3$).

Anal.—Calcd. for $C_{25}H_{33}BrO_5$: C, 60.85; H, 6.74. Found: C, 60.73; H, 6.72.

3β,14β-Dihydroxy-19-iodocarda-5,20(22)-dienolide 3-Acetate (VII)—A solution of 0.1 Gm. (0.00017 mole) of III and 0.1 Gm. (0.0007 mole) of lithium iodide in 20 ml. of isopropyl alcohol was heated under reflux for 2 hr. After evaporation of the solvent under reduced pressure, water was added to precipitate the product. Recrystallization twice from methanol gave 0.03 Gm. of product, m.p. 180–184°. Further recrystallizations from the same solvent gave the analytical sample, m.p. 184–185°; $[\alpha]_D^{19}$ −40° (c 0.6, $CHCl_3$).

Anal.—Calcd. for $C_{25}H_{33}IO_5$: C, 55.56; H, 6.16. Found: C, 55.79; H, 6.24.

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Determination of the Conformation of Nicotine and Some Related Compounds by Nuclear Magnetic Resonance and Dipole Moments

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A single NMR signal for protons in the 2- and 5-positions in *N*-methylpyrrolidine indicates rapid inversion of the nitrogen so that the methyl group spends half of its time on each side of the molecule. A single band for protons in the 2- and 5-positions in *N*-methylpyrrolidine *N*-oxide shows that the CH_3 and the O^- provide similar environments. Confirmation of this is obtained from the fact that NMR bands for comparable protons occupy nearly the same positions in *N*-methylpyrrolidine methiodide and *N*-methylpyrrolidine *N*-oxide. Introduction of a pyridine, or a pyridine *N*-oxide, ring in the 2-position causes the methyl protons and one proton on the asymmetric carbon to move upfield and all the other protons to move downfield. The upfield motion of these protons is consistent with the configuration which has the methyl group situated away from the pyridine ring. Dipole moments indicate that oscillation between two conformations is likely in (L)-nicotine and (L)-nicotine *N*-oxide, while (L)-nicotine *N*-oxide and (L)-nicotine *N,N'*-dioxide appear to be fixed in one preferred conformation.

ALTHOUGH the absolute configuration of natural (−)-nicotine is known (1), and nicotine *N*-oxide (compound *6* in Table I) is recognized as the first metabolite of nicotine in animals and plants (see *Reference 2*), no information is available concerning the conformation in which nicotine or its *N*-oxides exist. This problem has now been investigated by the use of techniques of nuclear magnetic resonance and dipole moment measurements.

Received October 3, 1966, from the Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, CA 94122
Accepted for publication January 19, 1967.
The authors wish to dedicate this paper to Dean T. C. Daniels, whose leadership at the University of California has done so much to raise the standards of education and research in the pharmaceutical sciences.
* Woodrow Wilson Foundation Fellow, 1961–1962. Abraham Rosenberg Fellow, 1962–1963. Trainee in pharmaceutical chemistry, 1963–1964, under grant 5T1-GM-728 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

DISCUSSION

Some previous work on the NMR spectrum of *N*-methylpyrrolidine (compound *1*) has been reported (3). The present results now make possible the unambiguous assignment of the methyl and methylene protons, and the effect of the conversion of the tertiary to a quaternized nitrogen, or to an *N*-oxide, has been observed to produce downfield shifts comparable to those reported in other compounds (3).

The relative downfield shift of the protons in *1* can be accounted for as follows. The *a* protons (Table I), being farthest away from the nitrogen, are shifted downfield the least amount. The others are