THE CARDIAC GLYCOSIDES OF *GOMPHOCARPUS FRUTICOSUS* (R.Br.)

IV. THE NUCLEAR MAGNETIC RESONANCE SPECTRUM OF GOMPHOSIDE

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Summary

Nuclear magnetic resonance spectroscopy supports the proposed structure (I) for gomphoside.

INTRODUCTION

Chemical evidence previously reported[1] resulted in structures (I) and (II) (see p. 576) being proposed for gomphoside and β-anhydrogomphogenin respectively. Other possible structures (e.g. III and IV) which were considered for gomphoside could be rejected on the basis of chemical evidence. Further evidence obtained from n.m.r. spectroscopy is now reported in support of the proposed structures.

DISCUSSION AND RESULTS

The n.m.r. spectra of gomphoside (I) (in deuterated pyridine), gomphoside acetate (V) (in deuterated chloroform and in pyridine-D(5)) and β-anhydrogomphogenin (II) (in $CDCl_3$) are listed in Table 1. Samples of digitoxin (VI) (in pyridine-D(5) and $CDCl_3$) digitoxigenin (VII) (in $CDCl_3$) and β-anhydrodigitoxigenin (VIII) (in $CDCl_3$) were measured to supply comparison n.m.r. spectra.

β-Anhydrogomphogenin

The spectrum of the genin, like that of β-anhydrodigitoxigenin and digitoxigenin, is considerably simpler than the corresponding glycoside. Two sharp three-proton peaks at 9·18 τ and 9·12 τ in β-anhydrogomphogenin, at 9·18 τ and 9·03 τ in β-anhydrodigitoxigenin, and at 9·15 τ and 9·08 τ in digitoxigenin can be assigned to the C(18) and C(19) methyl groups present in each compound.

The β-anhydrogomphogenin spectrum shows a single-proton doublet at 4·77 τ ($J = 2$ c/s) which is comparable to a similar doublet at 4·71 τ ($J = 2$ c/s) in β-anhydrodigitoxigenin, and which is absent in the digitoxigenin spectrum. This doublet can be assigned to the vinyl proton present in the D ring of β-anhydrodigitoxigenin, and the close similarity in the β-anhydrogomphogenin spectrum with respect to both field position and multiplicity strongly suggests that β-anhydrogomphogenin must also have a double bond in the five-membered D ring. Thus the Δ^{14} structure is confirmed as the correct one for β-anhydrogomphogenin.

Bands at 4·1 τ (area, one proton) and 5·2 τ (area, two protons), which are observed in the chloroform solution spectra of all the cardenolides studied, are

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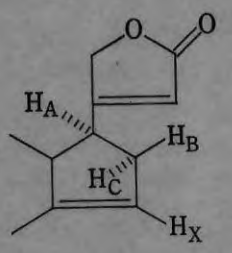
[1] Coombe, R. G., and Watson, T. R., *Aust. J. Chem.*, 1964, **17**, 92.

associated with the $\Delta^{\alpha\beta}$-butenolide system. The peaks which occur at 4·12 τ in β-anhydrogomphogenin, 4·08 τ in β-anhydrodigitoxigenin, and 4·14 τ in digitoxigenin appear as partially resolved multiplets (splitting = 1 c/s) and can be assigned to the vinyl proton on the α-carbon of the α,β-unsaturated lactone, split by long-range coupling. The peaks at 5·22 τ in β-anhydrogomphogenin, 5·20 τ in β-anhydrodigitoxigenin, and 5·10 τ in digitoxigenin are assigned to the two methylene protons of the butenolide ring.*

In β-anhydrogomphogenin a broad two-proton multiplet occurs between 6·2 τ and 6·8 τ. The high field value of much of this multiplet suggests that at least one of these protons may be axial.[2]

The spectrum of β-anhydrogomphogenin shows six protons between 7·0 τ and 7·7 τ. Two of these peaks are due to hydroxyl protons as a peak at 7·48 τ was reduced by an area of two protons when the compound was shaken with deuterium oxide. The remaining four protons can be assigned to the four allylic protons in the molecule at C(8), C(16) (two), and C(17) respectively. The region is complex, but a single-proton quartet centered at 7·20 τ is clearly visible. This quartet is probably due to the C(17) proton, which is the A proton of an ABCX system in which the A lines may be further broadened by allylic coupling with the butenolide vinyl proton H_Y.



Accurate analysis is not possible but by first-order analysis the following set of approximate values is obtained: $J_{AB} \doteqdot 9$ c/s, $J_{AC} \doteqdot 6$ c/s, $J_{BX} \doteqdot 2$ c/s, $J_{AX} \doteqdot 0$ c/s, $J_{CX} \doteqdot 1$ c/s, J_{BC} (small) ($\doteqdot 2$ c/s). $H_A = 7{\cdot}20$ τ, $H_B \doteqdot 7{\cdot}55$ τ, $H_C \doteqdot 7{\cdot}49$ τ, $H_X = 4{\cdot}77$ τ, $H_Y = 4{\cdot}12$ τ. β-Anhydrodigitoxigenin also shows four allylic protons between 6·95 τ and 7·75 τ, with a quartet at 7·13 τ due to H_A where $J_{AB} \doteqdot 10$ c/s, $J_{AC} \doteqdot 7$ c/s.

The remaining protons in the β-anhydrogomphogenin spectrum occur above 7·8 τ.

Gomphoside

The n.m.r. spectrum of gomphoside in pyridine solution shows two unsplit methyl peaks at 9·30 τ and 9·03 τ. Digitoxin in pyridine has methyl peaks at 9·10 τ and 8·98 τ, while gomphoside acetate in pyridine has peaks at 9·32 τ and 9·05 τ. The high field position of one of these methyl groups in gomphoside and its acetate in pyridine solution is probably caused by diamagnetic shielding from a nearby solvating pyridine molecule. Gomphoside acetate in deuterochloroform shows normal methyl peaks at 9·15 τ and 9·13 τ compared with values of 9·13 τ and 9·08 τ for digitoxin in chloroform, and so it is only in pyridine solution that the methyl peak of gomphoside and its acetate appear at high field values.

* Varian Associates in "N.M.R. at Work Series" No. 85 give 5·1 τ to these protons in digitoxigenin.

[2] Huitric, A. C., and Carr, J. B., *J. Org. Chem.*, 1961, **26**, 2648; McCasland, G. E., Furuta, S., Johnson, L. E., and Shoolery, J. N., *J. Amer. Chem. Soc.*, 1961, **83**, 2335.

Gomphoside and digitoxin show doublets at 8·61 τ ($J = 7$ c/s) and 8·68 τ ($J = 7$ c/s) respectively, owing to a methyl group, adjacent to an oxygen atom, being coupled to an adjacent proton. Such a system arises in digitoxose, the sugar linked to the aglycone in digitoxin, and the presence of doublets at these field values in the gomphoside spectrum confirms the presence of a 6-deoxy sugar residue in the gomphoside structure.

TABLE 1

N.M.R. SPECTRA

All field positions in τ values. B, broadened; D, doublet; FM, fine multiplet; M, multiplet; Q, quartet; S, singlet

Assignment	Gomphoside (I) in Pyridine-D(5)	Gomphoside Acetate		β-Anhydrogomphogenin in $CDCl_3$
		in $CDCl_3$	in Pyridine	
C(2), C(3)	5·5—6·0*	5·9—6·4†	5·5—6·0	6·2—6·8M
C(15)	‡	‡	—	4·77D ($J = 2$ c/s)
C(17)	7·25Q§	7·26QB§	7·25Q	7·20QB§
C(18), C(19) Me groups	9·03S, 9·30S	9·13S, 9·15S	9·05S, 9·32S	9·12S, 9·18S
C(21)	4·77FM	5·1—5·2	4·90	5·22D ($J = 2$ c/s)
C(22)	3·88BS	4·18BS	‖	4·12D ($J \doteqdot 1$ c/s)
C(1′)	4·53S	4·32S	4·73S	—
C(2′), C(3′) acetate	—	7·95S, 7·97S	7·92S, 7·92S	—
C(3′)	5·0—6·0*	5·1FM	5·6—6·0	—
C(5′)	5·0—6·0*	5·9—6·4†	5·6—6·0	—
C(6′)	8·61D ($J = 7$ c/s)	8·79 ($J = 6$ c/s)	8·68 ($J = 6$ c/s)	—

* A complex multiplet between 5·0 τ and 6·0 τ.
† A three-proton complex between 5·9 τ and 6·4 τ.
‡ Occurs with all other protons not mentioned above 7·45 τ
§ Couplings as described in text.
‖ Below 4·6 τ (obscured).

Apart from peaks in the gomphoside (pyridine-D(5) solution) spectrum at 3·88 τ (one proton) and 4·77 τ (two protons) due to the butenolide system, a further one-proton singlet was observed at 4·53 τ. This proton is assigned to the acetal proton at C(1) of the sugar residue where it is deshielded by two oxygen atoms and is in an environment with no protons on adjacent carbon atoms. The acetal proton in digitoxin is present at 4·56 τ (pyridine solution) and 4·99 τ (chloroform solution).

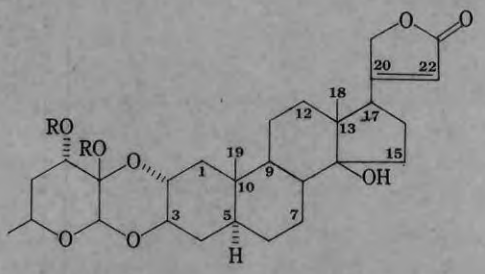
In addition gomphoside shows a quartet due to the allylic C(17) proton at 7·25 τ where $J_{16.17} \doteqdot 9$ c/s and $J_{16'.17} \doteqdot 5$ c/s.

Gomphoside Acetate

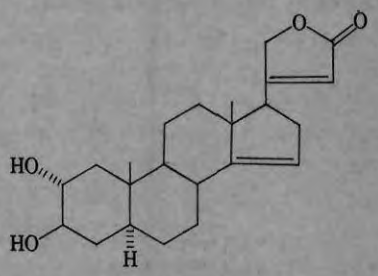
The changes involved in the n.m.r. spectrum by the acetylation of gomphoside may be summarized as follows:

(1) Two acetate groups at 7·95 τ and 7·97 τ indicate the formation of a diacetate and confirm the molecular formula.

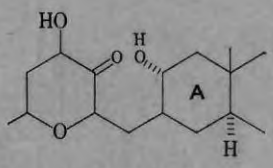
(2) Only one proton situated on a carbon also linked to an oxygen is materially affected by the acetylation; thus only one of the acetate groups is secondary. The other must be tertiary. Three protons attached to carbon also linked to oxygen occur



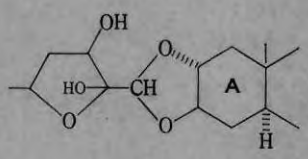
(I) R = H, gomphoside
(V) R = OAc, gomphoside acetate



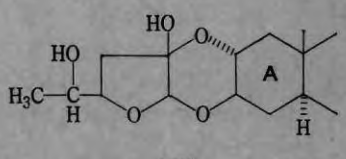
(II) β - anhydrogomphogenin



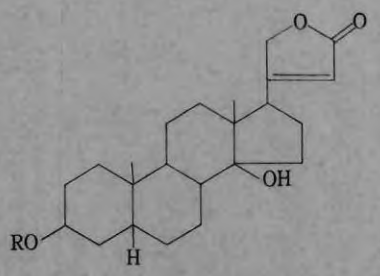
(III)



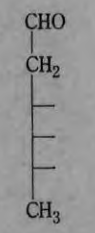
(IV)



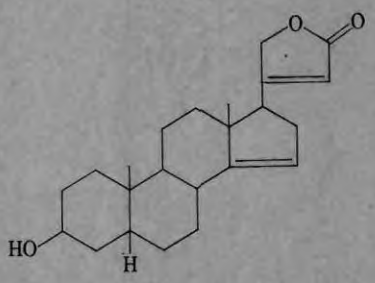
(IX)



(VI) R = (digitoxose)$_3$, digitoxin
(VII) R = H, digitoxigenin



digitoxose



(VIII) β - anhydrodigitoxigenin

between 5·9 τ and 6·4 τ, and the proton attached to the carbon carrying the acetate occurs at 5·1 τ where it is superimposed upon the butenolide methylene group. This evidence eliminates structure (III) which has two acylable secondary hydroxyl groups.

(3) A methyl doublet at 8·79 τ can again be assigned to the acetate of a methyl diamagnetically deshielded by an oxygen on the adjacent carbon. The coupling in this doublet (6 c/s) is not the same as that experienced by the proton attached to the carbon carrying the secondary acetate group ($J = 4$ c/s), and so the methyl group in the sugar residue is not adjacent to the acetate group. Thus the n.m.r. spectrum eliminates a structure such as (IX) for gomphoside.

(4) The acetylated compound showed the expected peaks for two unsplit methyl groups (9·13 τ and 9·15 τ), for the allylic C(17) proton (7·26 τ, quartet with further line broadening, $J_{16,17} \doteqdot 9$ c/s, $J_{16',17} \doteqdot 5$ c/s), for the butenolide vinyl proton (4·81 τ, singlet), and for the unsplit proton of the sugar acetal (4·32 τ).

Thus the n.m.r. spectra of the gomphoside series, when compared with digitoxin compounds of known structure, indicate that, apart from the glycosidic link, gomphoside has the characteristic cardenolide structure. Structures (III) and (IX) can be rejected on n.m.r. grounds, structure (IV) on chemical grounds,[1] and only structure (I) (without stereochemical implication) is supported by both n.m.r. and chemical evidence. Thus formula (I) is favoured for the structure of gomphoside.

Experimental

N.m.r. spectra were obtained at 60 m.c. and are measured relative to internal tetramethylsilane. The spectra of gomphoside, gomphoside acetate, and digitoxin were obtained in dilute pyridine-D(5) solution, and the spectra of gomphoside acetate, β-anhydrogomphogenin digitoxin, digitoxigenin, and β-anhydrodigitoxigenin were obtained in dilute $CDCl_3$ solution. β-Anhydrogomphogenin was deuterated by shaking in $CDCl_3$ solution with deuterium oxide.

Acknowledgments

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