[^{3}H]Clonazepam (16.2 Ci/mmol) was a gift of Hoffmann-La Roche (Basel, Switzerland). All other chemicals were of reagent grade and obtained from commercial suppliers.

Bovine brains were obtained from a local slaughterhouse and stored at −20 °C after dissection of the cortex. Membranes were prepared by homogenization in 10 vol of ice-cold 0.32 M sucrose containing protease inhibitors[10] in an Ultra-Turrax for 30 s. The homogenate was centrifuged at 1000*g* for 5 min at 4 °C and the supernatant was recentrifuged at 50000*g* for 30 min at 4 °C. The pellet was osmotically shocked by suspension in 20 vol of 50 mM Tris-HCl buffer at pH 7.4 containing protease inhibitors[10] and recentrifuged at 50000*g* for 30 min at 4 °C; the pellet was resuspended in 10 vol of 50 mM Tris-HCl buffer at pH 7.4.

The estimation of proteins was based on the method of Lowry[11] after membrane solubilization with 0.75 N NaOH. Bovine serum albumin was utilized as a standard.

Benzodiazepine receptor binding studies were performed by using a filtration technique and [^{3}H]flunitrazepam and [^{3}H]clonazepam as ligands. The membrane suspension (0.5 mg of protein) was incubated in triplicate together with approximately 0.9 nM [^{3}H]flunitrazepam or 0.9 nM [^{3}H]clonazepam and various concentrations of the displacers for 30 min at 0 °C in 500 μL of 50 mM Tris-HCl buffer at pH 7.4. After incubation the samples were diluted with 5 mL of assay buffer and immediately filtered under reduced pressure through glass fiber filter disks (Whatman GF/B) and then washed with 5 mL of the same buffer. Nonspecific binding was determined by parallel experiments containing diazepam (10 μM) and accounted for less than 10% of total binding.

Water-insoluble ester derivatives were dissolved in 50% ethanol/buffer and the same mixture was present in blank experiments.

The concentrations of the indole derivatives that inhibit specific [^{3}H]flunitrazepam binding by 50% (IC_{50}) were determined by log–probit analysis with four to six concentrations of the displacers, each performed in triplicate.

Registry No. 1, 94732-17-9; 2, 94732-18-0; 3, 94732-19-1; 4, 94732-20-4; 5, 94732-21-5; 6, 94732-22-6; 7, 94732-23-7; 8, 94732-24-8; 9, 94751-05-0; 10, 94732-25-9; 11, 94732-26-0; 12, 94732-27-1; 13, 94732-28-2; 14, 94732-29-3; 15, 94732-30-6; 16, 94732-31-7; 17, 94751-06-1; 18, 94732-32-8; 19, 94732-33-9; 20, 94732-34-0; 21, 94732-35-1; 22, 94732-36-2; 23, 94732-37-3; 24, 94732-38-4; 25, 94732-39-5; 26, 94732-40-8; 27, 94732-41-9; 28, 94732-42-0; 29, 94732-43-1; 30, 94732-44-2; 31, 94732-45-3; 32, 94732-46-4; 33, 94732-47-5; 36, 94732-50-0; 37, 94751-07-2; 38, 94732-51-1; 39, 94732-52-2; 40, 94732-53-3; 41, 94732-54-4; 42, 94732-55-5; 43, 94732-56-6; 44, 94732-57-7.

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Conformational Factors in Cardiac Glycoside Activity

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Gomphoside, a 5α-H cardiac glycoside isolated from *Asclepias fructicosa*, has an unique double glycosidic linkage to the aglycon through oxygen atoms at 2α and 3β of the steroid. The 3′-axial hydroxyl of its conformationally rigid sugar residue appears to be the functional group responsible for its potent inotropic activity. With use of gomphoside as the model compound, the conformation of the flexible glycosidic linkage of the 5β-H cardenolides, digitoxigenin α-L-rhamnoside and digitoxigenin β-D-digitoxoside, and the 5α-H cardenolides, uzarigenin α-L-rhamnoside and uzarigenin β-D-6-deoxyalloside, were investigated with the aid of computer graphics and conformational potential energy calculations. The relative inotropic potencies of these cardenolides can be accounted for by considering their active binding conformations with their potential energy distributions. The conformational distribution of the glycosidic moiety was postulated to be the major determinant of the biological activity of these cardenolides.

Cardiac glycosides are potent cardioactive agents that exert a positive inotropic effect unique to this class of compounds. Studies on the structure–activity relationships (SAR) in these compounds has long been a subject of interest and active research by many groups over many years.

It is generally accepted that an α,β-unsaturated lactone at 17β, a 14β-hydroxyl at the C/D cis ring junction, and a sugar residue at 3β of the C19 steroid nucleus are essential for optimal inotropic activity.[1] Introduction of extra hydroxyls to the aglycon generally reduces activity.

Gomphoside (I), first isolated in the Department of Pharmacy, University of Sydney,[2] was shown to have a unique chemical structure.[3,4] Apart from the A/B trans junction (where A/B cis is the more common configuration), the glycoside moiety is rigidly linked to the steroid through oxygen atoms at 2α and 3β of the steroid (Figure 1a). Several closely related cardenolides isolated later[5] were found to differ only in the configuration of substituent groups in the sugar moiety. The inotropic activity of these compounds was evaluated with use of isolated guinea pig atria, which is a commonly accepted technique that has been used by a number of groups to obtain comparable values for a wide variety of cardiac glycosides.[6-10] The results given (Table I)[11] are the potency of these compounds relative to digitoxigenin as the standard. The actual values, expressed as the molar concentration required to increase the force of contraction of the isolated

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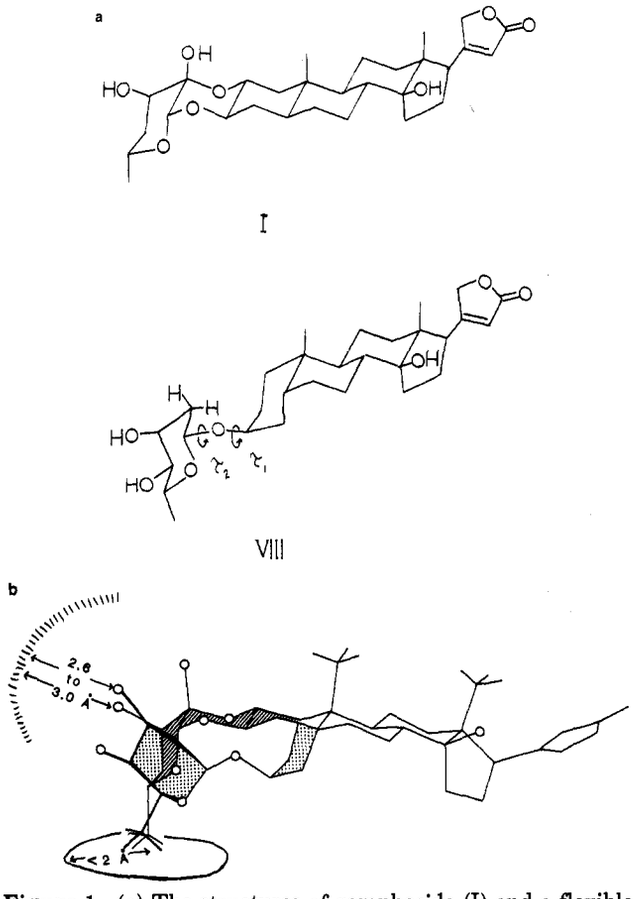

Figure 1. (a) The structures of gomphoside (I) and a flexible cardiac glycoside, digitoxigenin β-D-digitoxoside (VIII). (b) Computer graphics superimposed structures of gomphoside (I) (shaded part) and digitoxigenin α-L-rhamnoside (VII) in its proposed binding conformation (dotted part) showing the hypothetical binding area for the hydrogen-bonded hydroxyl and the hydrophobic binding of the 5′-methyl.

Table I. Relative Inotropic Activity of the Gomphoside Series (ΔF_{75} Digitoxigenin = 1×10^{-6} M)

	COMPOUND	STRUCTURE	RELATIVE INOTROPIC ACTIVITY [a]
I	GOMPHOSIDE		23
II	DIDEHYDRO GOMPHOSIDE		2·5
III	EPI-GOMPHOSIDE		0·2
IV	4′-HYDROXY GOMPHOSIDE		1
VI	DIGITOXIGENIN		1

[a] ΔF_{75}, GUINEA PIG LEFT ATRIA. DIGITOXIGENIN = 1

atrium by 75% (ΔF_{75}), for gomphoside is 6.2×10^{-8} M and for digitoxigenin 1.4×10^{-6} M. (The inotropic activities for the compounds in Tables II and III are taken from ref 9 and 10.)

Table II. Relative Inotropic Activity of the 5β Series (ΔF_{75} Digitoxigenin = 1×10^{-6} M)

	COMPOUND	STRUCTURE	RELATIVE INOTROPIC ACTIVITY [a]
VII	DIGITOXIGENIN-α-L-RHAMNOSIDE		22
VIII	DIGITOXIGENIN-β-D-DIGITOXOSIDE		15
VI	DIGITOXIGENIN		1

[a] ΔF_{75}, GUINEA PIG LEFT ATRIA. DIGITOXIGENIN = 1

Table III. Relative Inotropic Activity of the 5α Series (ΔF_{75} Digitoxigenin = 1×10^{-6} M)

	COMPOUND	STRUCTURE	RELATIVE INOTROPIC ACTIVITY [a]
X	ASCLEPOSIDE UZARIGENIN-β-D-6′-DEOXYALLOSIDE		3
IX	UZARIGENIN-α-L-RHAMNOSIDE		9
VI	DIGITOXIGENIN		1
V	UZARIGENIN		1·2

[a] ΔF_{75}, GUINEA PIG LEFT ATRIA. DIGITOXIGENIN = 1

Since gomphoside is one of the most potent cardiac glycosides known, and since the potency of the compounds in Table I is progressively reduced by changing the configuration of the 3′-oxygen functional group from the 3′-axial hydroxyl (I), to the 3′-ketone (II), and the 3′-equatorial hydroxyl (III), it is apparent that the 3′-axial hydroxyl of gomphoside, and in particular, its location with respect to a particular binding site in the receptor, contributes to its very high activity. Inotropic activity was also reduced by acetylation of the 2′- and 3′-hydroxyls, or by acetonide formation on the cis glycol, and by the introduction of a 4′-equatorial hydroxyl (IV) to the structure of gomphoside.[11] It has also been oberved that 6-deoxyhexosides are more potent than those glycosides containing a terminal hydroxymethyl group. Thus, with use of the conformationally rigid and highly potent gomphoside as a model of the configurational and conformational requirements in cardiac glycosides that are necessary for maximal positive inotropic activity, it is proposed that as well as the binding site for the 3′-axial hydroxyl there is

a broad hydrophobic region in the receptor which accommodates the α-face of the hexose and the 5′-methyl. Hence, in those glycosides which have a 6-deoxy sugar residue, the 5′-methyl should be directed preferably toward this region.

The hypothesis that is being explored is that there are two primary binding sites in the receptor for the sugar moiety, one a hydrogen bonding site located in a volume determined by the 3′-hydroxyl group of gomphoside and the other a broad hydrophobic region where the 5′-methyl group binds by van der Waals forces. If those glycosides which have singly bonded carbohydrate residues, i.e., those which are conformationally flexible, can take up low-energy conformations which at the same time allow their respective terminal methyl groups and at least one hydroxyl to fit the binding regions defined by those corresponding groups of gomphoside, then it could be expected that those glycosides would have inotropic activities comparable with that of gomphoside (Figure 1b).

Discussion

Research into SAR of the glycosidic moiety of cardiac glycosides has been directed mainly toward the effect of the various functional groups.[9,12-17] The 3′-hydroxyl[12,15] and the 4′-hydroxyl[9] were considered to be the most important for hydrogen bonding.

In this study, a new approach has been taken to explore the variations in inotropic potencies which cannot be explained by the subtle differences in the configuration of the glycosidic functional groups of the otherwise structurally very similar cardiac glycosides. Although the presence of the glycosidic moiety in a cardenolide increases the inotropic activity compared to the corresponding aglycon, in general the maximal enhancement is only about 20-fold (e.g., the increase in potency of digitoxigenin α-L-rhamnoside (VII) compared to the aglycon digitoxigenin (VI) is about 23 times (Table II)). On the contrary, the variation in the aglycon structure can change the inotropic activity by several orders of magnitude (e.g., $2\alpha,15\beta$-dihydroxyuzarigenin, afrogenin,[9] is more than 100 times less potent than uzarigenin (V)). Thus, it is apparent that the steroid aglycon provides the major part of the binding energy to the receptor, whereas the glycosidic portion plays a secondary role in stabilizing the cardenolide–receptor complex. However, in cases when the steroid aglycons are similar, the conformational factors of the flexible glycosidic moiety are proposed to play an important role in determining the inotropic activities. Working on the assumption that all the glycosidic hydroxyl groups responsible for receptor binding in different cardiac glycosides share a common hydrogen bonding site, the probable binding conformations of the glycosidic moiety of the different cardiac glycosides have been investigated by using computer graphics. A qualitative picture of the relative conformational distribution is then obtained by using a semiempirical classical potential energy calculation in an attempt to rationalize the differences in biological activity.

Gomphoside (I) has the same steroid structure as the conventional cardenolides at the BCD ring region, but its A/B ring junction is trans. It has been shown that 5α-H and 5β-H cardenolide aglycons, such as uzarigenin (V) and digitoxigenin (VI), respectively, have very similar inotropic activities,[9,10] which implies that at least in this test preparation this stereochemical difference has very little effect on the biological activity. Thus, it appears that the major reason for the high biological activity of gomphoside is the glycosidic part of the molecule and, in particular, the proximity of the 3′-hydroxyl to the hydrogen bonding site of the receptor.

For cardiac glycosides with the normal single glycosidic linkage, the position in space of the hydroxyls on the sugar residue is variable, due to the two degrees of rotational freedom about the glycosidic linkage (Figure 1a). It can be shown by using computer modelling that most of these hydroxyls can be rotated into a position of close proximity to the hypothetical hydrogen bonding site as defined by the fixed conformation of gomphoside. Thus, all cardiac glycosides are more potent than the corresponding aglycons due to this additional hydrogen bonding, but all of the glycosides with a higher order of potency have a 6-deoxyhexose residue, suggesting that there is a hydrophobic binding site for the 5′-methyl group or alternatively that the presence of a polar function at this region is undesirable. In either case, a second constraint is imposed on the choice of probable binding conformations of the sugar residue in addition to the hydrogen bonding.

Since the binding of the steroid aglycon is accepted to be the initial step of the cardenolide–receptor interaction,[15] the probable binding conformations of the glycosidic moiety of these cardiac glycosides can be deduced with the aid of computer graphics and distance of separation calculations, by first superimposing the aglycon part of the flexible cardenolide with that of gomphoside, the model compound. The two variable torsional angles (τ_1, C_2–C_3–O_3–$C_{1'}$; τ_2, C_3–O_3–$C_{1'}$–$C_{2'}$) were changed until an optimal degree of superimposition between the methyls ($C_{6'}$) and the hydroxyls ($O_{3'}$) of the two cardenolides were reached. The variation of the separation between the superimposing pairs of functional groups, for instance, the 5′-methyl vs. 5′-methyl and the 3′-hydroxyl vs. 3′-hydroxyl, of gomphoside and the flexible cardenolide, digitoxigenin α-L-rhamnoside, respectively, can be represented as a function of the two variable torsional angles, τ_1 and τ_2, in a contour diagram (Figure 2).

The binding conformations of a flexible glycoside are considered possible when the methyl and the hydroxyl are separated from the corresponding functional groups of gomphoside with the following criteria. For the methyl group, a maximum deviation of 1 to 2 Å is allowed, since if this is a specific hydrophobic binding region, then a methyl located further than 2 Å is not likely to bind. For hydrogen bonding to occur, any one of the hydroxyl groups of the sugar residue must be located within an optimal hydrogen bonding distance from the receptor site, namely, 2.6 to 3.0 Å.[18] Conversely, the hypothetical binding site can be positioned anywhere around the hydroxyl oxygen at the binding position but within 2.6–3.0 Å, taking into account the hydrogen-bond vector. Thus, the possible location of this binding site relative to the bound gomphoside can be first limited to the volume enclosed by two concentric hemispheres, with radius 2.6 and 3.0 Å, respectively, and with $O_{3'}$ of gomphoside as the center, and pointing away from the $C_{3'}$–$O_{3'}$ bond axis. For the other hydroxyl groups of the flexible cardiac glycosides to be able

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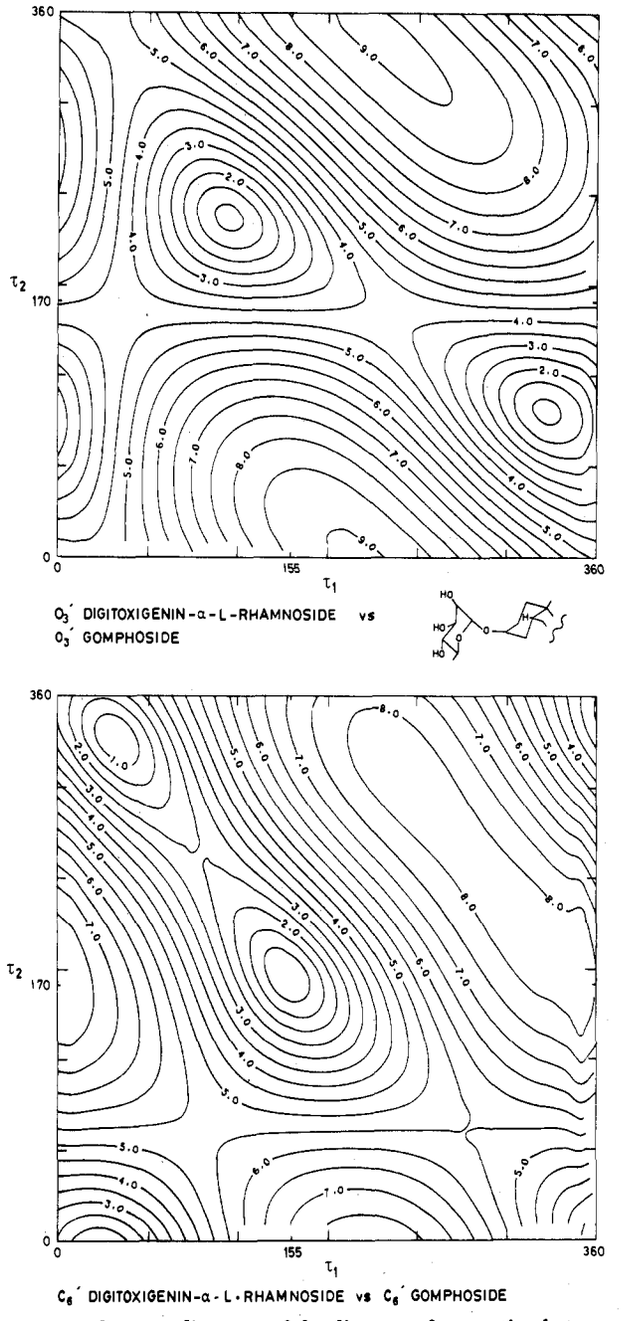

Figure 2. Contour diagrams of the distance of separation between gomphoside (I) and digitoxigenin α-L-rhamnoside (VII) (see Experimental Section).

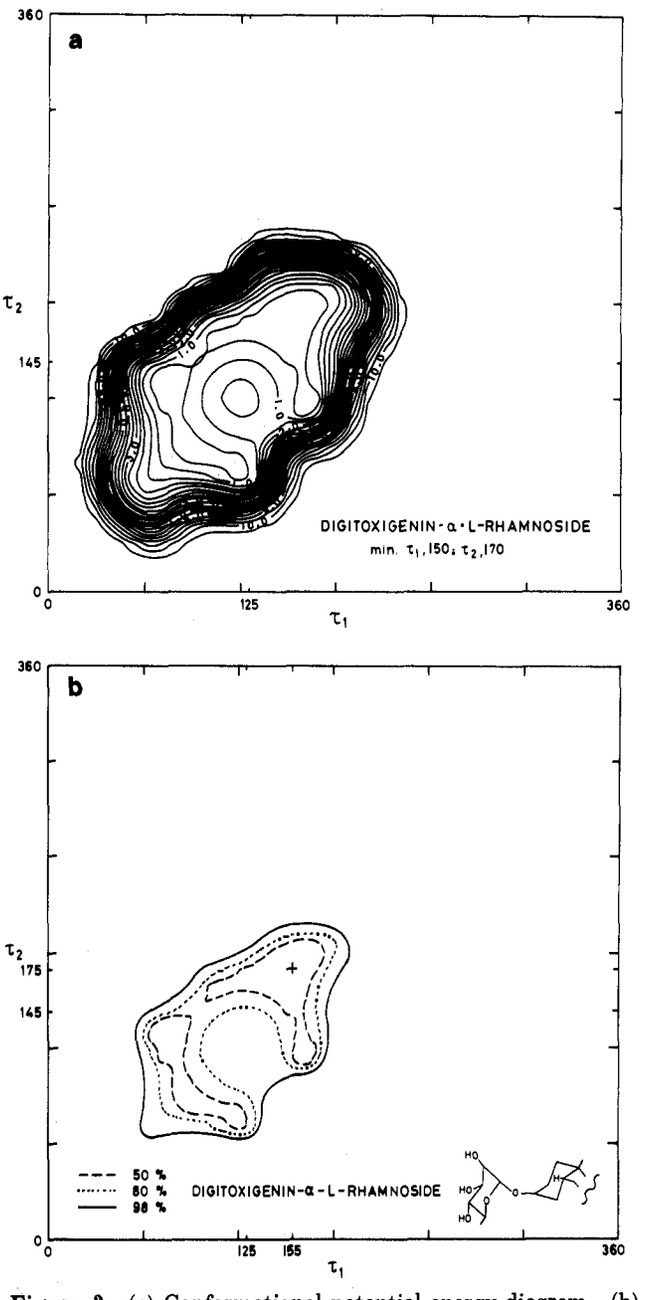

Figure 3. (a) Conformational potential energy diagram. (b) Population distribution diagram of digitoxigenin α-L-rhamnoside (VII) (see Experimental Section).

to share the same hydrogen bonding site with gomphoside, the possible hydrogen-bonding region described for a particular hydroxyl must have a common overlapping volume with that of the 3′-hydroxyl of gomphoside when the aglycon parts are superimposed. Thus, taking into account the C–O bond vector, theoretically the hydroxyl of the flexible glycoside can be up to 4–5 Å away from the position of the $O_{3'}$ of gomphoside (Figure 1b). Thus, when the potential energy is considered at the same time, a probable range of binding conformations can be postulated. Digitoxigenin α-L-rhamnoside (VI) and digitoxigenin β-D-digitoxoside (VIII) are examples that satisfy the requirements stated above. Compound VII and gomphoside (I) are equipotent, but VIII is only about 60% as active as VII (Table II). Since both VII and VIII have the two binding requirements, the difference must be related to the distribution of the conformational isomers of the glycosidic residue.

From conformational potential energy calculations, it can be shown that the rotational freedom of VII is more restricted than that of VIII (Figures 3a and 4a), and the conformation of the proposed binding configuration of VII (τ_1, 155°; τ_2, 170°) lies well within the global energy minimum. On the contrary, the proposed binding conformation of VIII (τ_1, 70°, τ_2, 260°) was located on the side of the energy well.

Theoretically, the population of each conformer of the cardiac glycoside in solution, before binding to the receptor occurs, is dependent on its potential energy relative to the energy distribution of all of the other possible conformers. If the conformational potential energy is calculated in reasonably small rotational intervals, a population distribution map can be generated with use of the Boltzmann's distribution function,[19] viz., probability, $Z_{ij} \propto$

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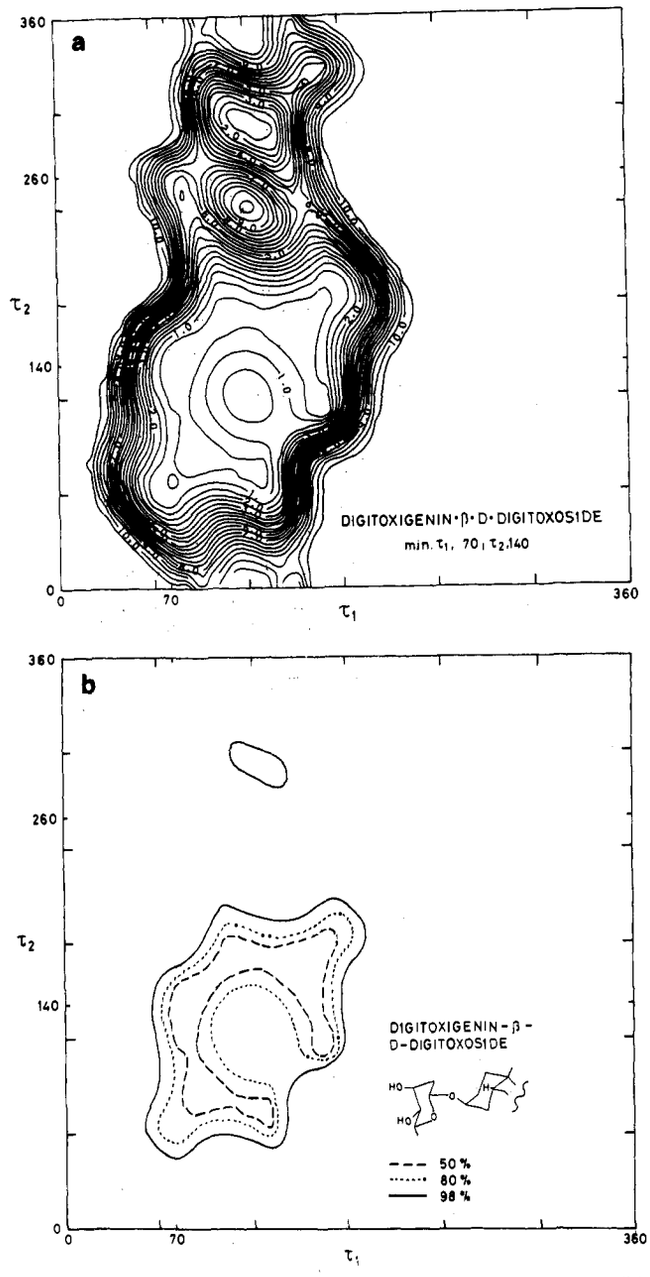

Figure 4. (a) Conformational potential energy diagram. (b) Population distribution diagram of digitoxigenin β-D-digitoxoside (VIII) (see Experimental Section).

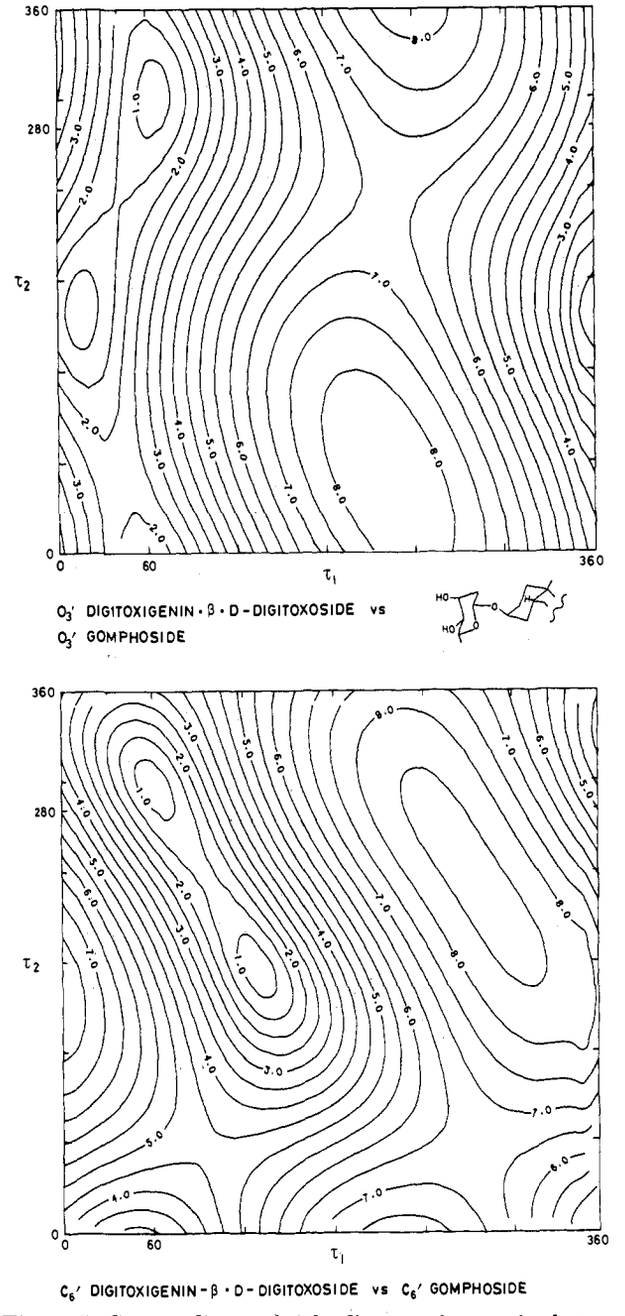

Figure 5. Contour diagrams of the distance of separation between gomphoside (I) and digitoxigenin β-D-digitoxoside (VIII) (see Experimental Section).

exp($-\Delta E_{ij}/RT$), and normalized to the whole conformational space (Figures 3b and 4b).

The equilibrium distribution of the cardiac glycoside in the favorable binding conformation in solution, which is governed by the conformational energy distribution, represents the probability of the receptor coming into contact with the favorable binding species among all the other unfavorable conformers. Thus, compounds with their binding conformation similar to the preferred conformation but with a restricted rotational freedom should have a higher probability of binding to the receptor relative to those with a wider rotational freedom or with the binding conformation at a higher energy level.

Digitoxigenin α-L-rhamnoside (VII) has a restricted rotational freedom which is defined by a single deep potential energy minimum (Figure 3a). This feature essentially allows VII to behave in solution as a single conformational entity since all the conformers within this minimum are closely related and so readily interconvert. At the hypothetical binding conformation (τ_1, 150–160°; τ_2, 170–180°), the 5′-methyl is separated by less than 1 Å from the 5′-methyl of gomphoside, and the 3′-hydroxyl about 3–3.5 Å, and the 4′-hydroxyl about 2–2.5 Å from the 3′-hydroxyl groups of gomphoside (Figure 2). With the proposed binding conformation coincidental with the energy minimum, the binding affinity will only be determined by the degree of fitness of the binding species to the conformer. This is analogous to the rigid compounds I–IV existing entirely as a single species in solution, in which the degree of fitness to the inotropic receptor dictates their inotropic activities.

On the other hand, digitoxigenin β-D-digitoxoside (VIII) has a much wider rotational freedom with two major potential energy minima (Figure 4a). Thus, VIII exists as an equilibrium mixture of two or more sets of conformers in solution. The ideal binding conformation of VIII is at τ_1, 50–70°, τ_2, 280–310°, where both of the 5′-methyl and

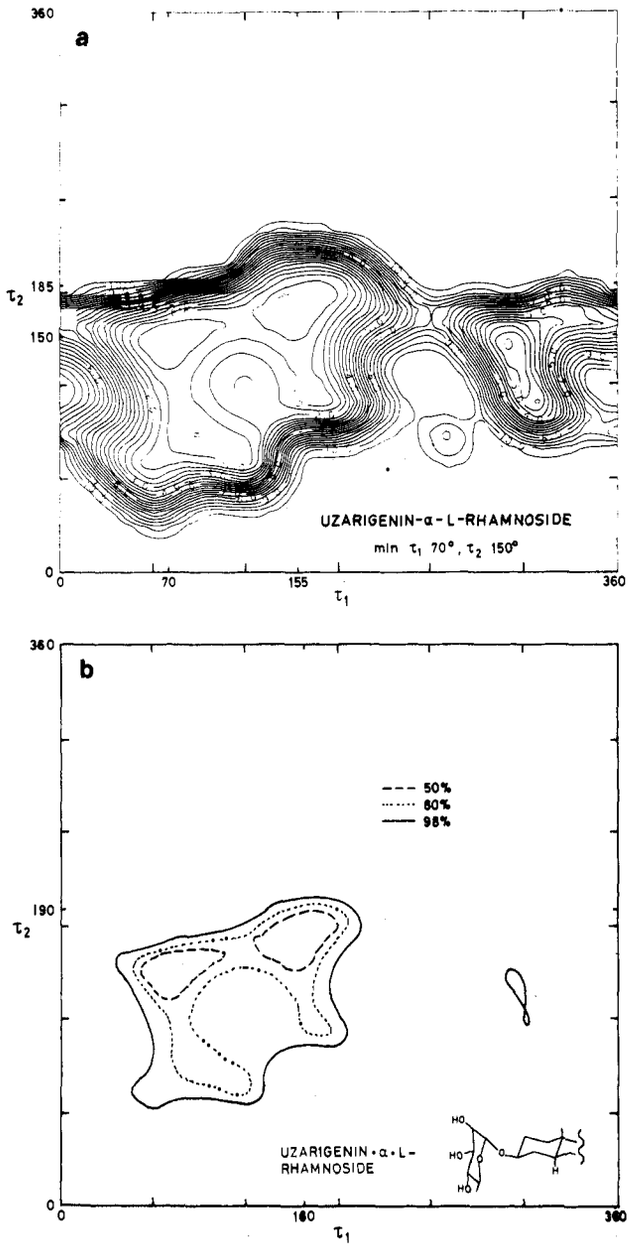

Figure 6. (a) Conformational potential energy diagram. (b) Population distribution diagram of uzarigenin α-L-rhamnoside (IX) (see Experimental Section).

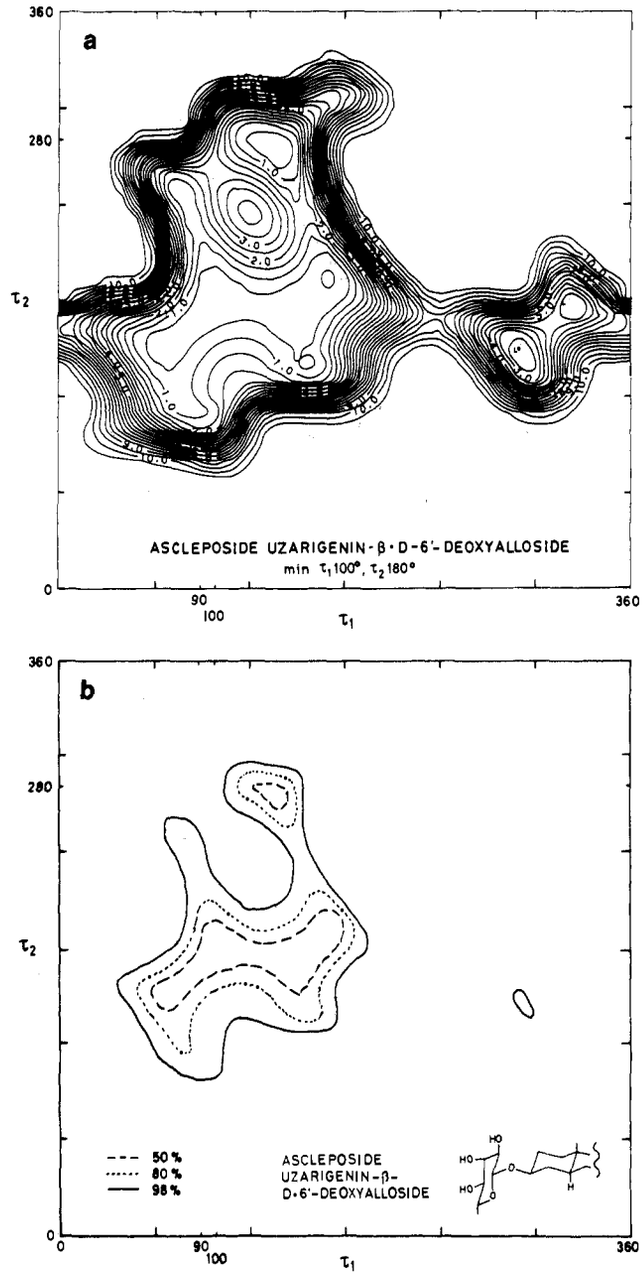

Figure 7. (a) Conformational potential energy diagram. (b) Population distribution diagram of uzarigenin β-D-6-deoxyalloside (X) (see Experimental Section).

3'-hydroxyl are within 1 Å of the corresponding functional groups of gomphoside. However, due to the fact that in this conformation, the α-hydrogen atom at 2' is less than 2 Å from those of the A ring, the potential energy (ca. 100 kcal/mol) is too high to allow this conformation to be feasible as the binding conformation. At τ_1, 60–80°, τ_2, 250–270°, the 5'-methyl is about 1–2 Å from the 5'-methyl of gomphoside and the 3'-hydroxyl 2–3 Å and the 4'-hydroxyl 3–3.5 Å away from the 3'-hydroxyl group of gomphoside (Figure 5). With a potential energy of about 6 kcal/mol, this is acceptable as a feasible binding conformation. Since the hypothetical binding conformation for VIII is located on the high-energy side of the global minimum, the proportion of VIII with this conformation in solution will be considerably lower than in the case of VII, as well as the binding will be energetically less favorable. Although the degree of fitness of VII and VIII to the receptor in the respective binding conformations may be similar, VIII is less active (about 70% as potent as VII, Table II[10]).

Similarly, for the 5α-H cardiac glycosides, uzarigenin α-L-rhamnoside (IX) and uzarigenin β-D-6-deoxyalloside (ascleposide) (X), the same relationship is observed. At the hypothetical binding conformation of IX, (τ_1, 150–160°; τ_2, 180–190°), the 5'-methyl is separated by less than 1 Å from the 5'methyl of gomphoside, and the 3'-hydroxyl about 3 Å, and the 4'-hydroxyl about 2 Å from the 3'-hydroxyl group of gomphoside, with potential energy of less than 1 kcal/mol (Figure 6). For X, the 5'-methyl is less than 1 Å from the 5'-methyl of gomphoside, and the 3'-hydroxyl and 4'-hydroxyl about 2–3 Å from the 3'-hydroxyl group of gomphoside, with potential energy of about 3 kcal/mol (Figure 7). Since the rotational freedom of (IX) is more restricted, the probability of IX binding to the receptor and hence its inotropic potency is higher than X. Thus, IX is about 3 times as potent as X (Table III).[9]

Digitoxigenin α-L-rhamnoside (VIII) and uzarigenin α-L-rhamnoside (IX) differ only at the A/B ring junction.

When their conformational energy maps are compared (Figures 3 and 6), it is apparent that the A/B trans junction provides a higher degree of rotational freedom. Since there would be two major groups of conformers in relation to the two energy minima in IX, instead of one in VII, the probability of binding and the observed inotropic potency of IX are lower. Thus, IX is about 40% as potent as VII.

Furthermore, it would be reasonable to propose that it is this conformational factor that rendered the A/B trans cardenolide glycosides less potent than the A/B cis glycosides in general rather than the steric requirement for the cis A/B junction commonly advocated by most other research groups.

Conclusion

By use of this approach, the order of potency of each pair of cardiac glycosides can be accounted for by the fact that, kinetically, the effective concentration of those with more restricted rotations is higher although their optimal fitness to the receptor may be similar.

The important distinction between the preferred conformation of a biologically active compound in solution before binding to the receptor occurs and the actual conformation that it adopts in the drug–receptor complex should be noted. For the highly active compounds, the preferred conformation should be very similar to the binding conformation, whereas the binding conformation of the less active ones would be energetically less favorable or be within a minor population of conformers.

It should also be pointed out that the method used for PE calculation might not be able to yield very accurate energy values; however, the relative potential energy values (ΔE) obtained for a series of closely related compounds would still be able to give a reasonable approximation of the relative conformational potential energy in order to produce a qualitative measure of the relative population distribution.

With the advance of computer modelling, the conformational aspect of SAR can be handled in a more precise manner, enabling the prediction of the probable binding conformation. When used in conjunction with potential energy calculations, a rational approach to the study of the conformational factors in SAR is achieved.

Experimental Section

Inotropic activity was measured with use of isolated guinea pig left atria.[13]

Molecular modelling and superposition were carried out by using an interactive graphics program, CRYSX, running on an Evans and Sutherland picture system supported by a PDP 11/34 computer at the UCC, Sydney. The molecules examined were constructed from published crystallographic data of aglycons and sugars. The crystal structure of gomphoside was determined by X-ray crystallography.[20]

Conformational energy calculations were carried out by using a classical potential energy program, COMOL,[21,22] at 10° intervals for the two variable torsional angles, τ_1, C_2–C_3–O_3–$C_{1'}$, and τ_2, C_3–O_3–$C_{1'}$–$C_{2'}$. Since there is still no universally accepted method to satisfactorily account for the effect of solvation, the effect of hydration of the molecules in solution has been neglected.

The population distribution calculation, carried out with use of the ΔE values from the conformation energy calculation, is based on the Boltzmann's distribution function.[19] The probability of existence of each state or conformer is proportional to the term $\exp(-\Delta E_{ij}/RT)$, where ΔE_{ij} is the potential energy of the conformer, R the gas constant, and T the absolute temperature.

The Z_{ij} values are normalized to the whole conformational space. The cumulative population distribution is then calculated by integrating Z_{ij} above a computed isoprobability value such that each contour represents a boundary which encloses the specified proportion of the most likely conformers (or alternatively, the chance of finding a molecule with the conformation enclosed by this boundary at any one time).

The distance of separation calculations were carried out by defining the geometry of the superimposing functional groups (3′-hydroxyl, 5′-methyl) of gomphoside in the coordinate frame of the flexible cardiac glycoside when the aglycon part of the two cardenolides were optimally superimposed. The distances between each pair of the superimposing functional groups was computed at each 10° step of rotation about τ_1 and τ_2.

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Synthesis and Dopaminergic Activity of (*R*)- and (*S*)-4-Hydroxy-2-(di-*n*-propylamino)indan

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A synthetic precursor to a potent dopaminergic agonist, (*RS*)-4-hydroxy-2-(di-*n*-propylamino)indan (1), has been resolved by classical recrystallization procedures, and the absolute configurations of the enantiomers have been established by X-ray crystallographic analysis. The enantiomers were converted by literature procedures into (*R*)- and (*S*)-1. (*R*)-1 was approximately 100 times as potent as (*S*)-1 in an assay for dopamine agonist effect in the isolated cat atrium.

Hacksell et al.[1] reported powerful dopamine agonist effects for (*RS*)-4-hydroxy-2-(di-*n*-propylamino)indan (1). The present structure–activity study was aimed at resolution of (*RS*)-1 and investigation of the stereochemistry of this dopaminergic agent. Our preparation of 1 differed from the literature method[1] and is shown in Scheme I.

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