EXPERIENTIA

Vol. 33 – Fasc. 6 Pag. 697–836 15. 6. 1977

GENERALIA

Cardiac glycosides: Prerequisites for the development of new cardiotonic compounds

by Th. W. Güntert and H. H. A. Linde[1]

Pharmazeutisches Institut der Universität Basel, Totengässlein 3, CH–4051 Basel (Switzerland)

Summary. Two prerequisites in a successful search for cardiac glycosides are the separability of the inotropically-active from the toxically-active component and knowledge of their structure-activity relationships and/or indispensible structural features. A number of papers show that toxic effects of heart glycosides are dependent upon inhibition of the Na^+, K^+-transport ATPase. This is not, however, the case with the inotropic effect, which has been shown not to be causally related to inhibition of the ATPase. Thus it is reasonable to assume that the search for selectively active compounds with the digitalis effect will be met with success. At present, however, there are no assumptions which can be made about the dependence of the pharmacological effect upon the structure. This would of course allow the prediction of structural details of the effective inotropic substance, and thus make possible its synthesis. Various studies have disproved the dogmas which have been adhered to for a long time. The structure-activity relationships are still obscure, although a host of individual phenomena are known. Some insight, however, results from those cases in which either the animal therapeutic index of this class of compounds is increased, or the frequency of arrhythmias is diminished. In elucidating such properies, the array of test methods used is of great importance. Methods, frequently applied today, such as the one determining the toxicity in the cat (Hatcher) or the ATPase test can easily lead to wrong conclusions unless they are used in conjunction with more revealing tests for inotropy.

The difficulties and risks of therapy with cardiac glycosides have as yet not been overcome. This is because of the narrow therapeutic spectrum[2,3], as well as wide differences in patient responses to this class of compounds[4–6]. Although the search for natural or synthetic substances with improved therapeutic properties has been pursued intensively, it has thus far not produced satisfactory results. 2 prerequisites for success of such attempts are the knowledge of the structure-activity relationships of such molecules, as well as the separability of therapeutic from toxic effects. It is possible to separate these 2 effects only if either 2 different mechanisms or 2 different receptors are responsible for the effects.

Separability of therapeutically-desirable and toxic digitalis effects

Although the search for the mechanism of cardiac glycoside effects is far from complete[7,8], there are increasing indications that cardiotonic and cardiotoxic effects need not necessarily be correlated. According to Repke's theory, the biochemical mechanism for both therapeutic and toxic effects of heart glycosides is based on an inhibition of a single enzyme[6,9]. That the arrhythmias induced by digitalis are partly due to the inhibition of the Na^+, K^+-activated ATPase in the cell membrane, remains undisputed[10–16]. Okita et al., however, have succeeded in excluding a causal relationship between enzyme inhibition and positive ino-

1 Acknowledgment. The authors are very grateful to J. Berger and Dr M. Sayare for their kind help and advice in writing the English version of the manuscript, as well as for support by the Swiss National Science Foundation.

2 W. Diederen and R. Kadatz, Ärztl. Forsch. *24*, 149 (1970).
3 M. A. Kimble and R. M. Elenbaas, J. Am. pharm. Ass. *14*, 362 (1974).
4 Cardiac Glycoside Therapy, in: Evaluations of Drug Interactions, p. 295. American Pharmaceutical Association, Washington, 1973. 1st ed.
5 G. K. Moe and A. E. Farah, in: The Pharmacological Basis of Therapeutics, 5th ed., p. 653. Ed. L. S. Goodman and A. Gilman. Macmillan Publ. Co. Inc, New York 1975.
6 K. Repke, Internist *7*, 418 (1966).
7 K. S. Lee and W. Klaus, Pharmac. Rev. *23*, 193 (1971).
8 T. Godfraind, Biochem. Pharmac. *24*, 823 (1975).
9 K. R. H. Repke, R. Schön, W. Henke, W. Schönfeld, B. Streckenbach and F. Dittrich, Ann. N. Y. Acad. Sci. *242*, 203 (1974).
10 R. E. Ten Eick, A. L. Bassett and G. T. Okita, J. Pharmac. exp. Ther. *185*, 12 (1973).
11 G. T. Okita, F. Richardson and B. F. Roth-Schechter, J. Pharmac. exp. Ther. *185*, 1 (1973).
12 G. T. Okita, Proc. West Pharmac. Soc. *18*, 14 (1975).
13 R. Mendez, G. Pastelin and E. Kabela, J. Pharmac. exp. Ther. *188*, 189 (1974).
14 H. Lüllmann and T. Peters, Adv. Cardiol. *12*, 174 (1974).
15 T. Peters, R.-H. Raben and O. Wassermann, Eur. J. Pharmac. *26*, 166 (1974).
16 B. G. Katzung, J. A. Munoz, D. Y. Shirachi, A. J. Trevor, H. H. Chang and M. E. Wolff, Experientia *26*, 1189 (1970).

tropy[11]. Using isolated Langendorff perfused rabbit heart preparations, they were able to show that the time needed to wash out the inotropic effect, does not coincide at all with that needed to wash out the enzyme inhibition. In fact, after all inotropic effect was washed away, the tissue still showed enzyme inhibition of up to 40%. (Okita is one of the few who measures inotropy and ATPase inhibition in the same biological material.) A comparison of the half-life for the washout of the 2 effects after strophanthidin-3-bromoacetate perfusion clearly shows the separation: $t_{1/2}$ for the positive inotropic effect is approx. 4 min, whereas $t_{1/2}$ for the enzyme inhibition extends to several hours. Also, for the glycoside ouabain, they find a marked difference in the period needed to wash out the 2 effects[11]. This was further confirmed by Akera et al.[17] using somewhat different time scales. (The stability of the glycoside-ATPase-complex depends on the duration of incubation of the glycoside with the enzyme[11]. The formation of the complex seems to be dependent on ATP[18].) In accordance with the results of Okita, Lüllmann et al.[14] have been able to demonstrate the extreme stability of the ouabain-ATPase-complex. They too noted a striking discrepancy between the rapid disappearance of ouabain-induced inotropy in washout experiments and the strong binding of heart glycosides to the transport enzyme.

Kinetic studies are not the only source of data making it improbable that a causal relationship exists between Na^+, K^+-ATPase inhibition and the positive inotropic effect. Quinidine, oligomycin, sulfhydryl group blocking agents and sodium azide blocked the Na^+, K^+-transport enzyme in various experiments, but none of these substances exert a positive inotropic effect[7]. Furthermore it has been demonstrated by several authors[15, 19] that there is no dependence of the positive inotropic effects of a number of cardiotonic compounds on their efficiency to inhibit the transport enzyme. This is additional evidence supporting the absence of a direct connection between ATPase inhibition and inotropy. Moreover Murthy et al.[20] have shown that with a number of digitalis steroids, a given percentage of inhibited enzyme does not coincide with a single inotropic potency. But according to Repke's postulated[6] and still advocated[21] mechanism in which the extent of enzyme inhibition determines the presence of therapeutic and/or toxic effects, such a dependence should exist. Results of Lüllmann et al.[22, 23] also fail to support such a connection. These authors were able to show that metabolites of digitoxin and digoxin were exclusively inotropic in effect, in doses which normally are accompanied by toxic effects. On the other hand, Katzung et al.[16] demonstrated that the inotropic effect of cardenolides on left atria of guinea-pigs can be prevented or delayed by preliminary treatment with acetylisodigitoxigenin or acetylisodigitoxigenic lactam, whereas the inhibitory effect of the cardenolides on the Na^+, K^+-ATPase as well as the toxic signs in the ECG are fully retained.

All these observations point to the fact that the cardenolide effect on the transport enzyme and on the power of contraction can be uncoupled, and furthermore that the inhibition of the Na^+, K^+-ATPase by digitalis is responsible for the toxic but not for the inotropic effect. With all these indications, however, it is still an unanswered question how the amount of free Ca^{2+}-ions is increased at the contractile proteins under the influence of digitalis in inotropic doses. Promising first steps to a solution of this problem result from the work done by Langer et al.[24, 25] and Nayler[26], who found that La^{3+} can displace considerable amounts of Ca^{2+} from the cell surface. Under its influence, no contraction develops, though an action potential can still be measured. Digitalis enhances this displacement effect from superficially located stores, the amount of free Ca^{2+} is increased.

Test methods

The effectiveness of various cardiotonic compounds is, strictly speaking, only comparable if all such compounds are tested under the same conditions. However, as can be seen from the literature, this is by no means the case. Thus the validity of the structure-activity relationships quoted in the next section is limited. The information inherent in a test method should always be critically assessed. Results using methods which are based on a model (e.g. ATPase test), or in which the desired effect is not directly measured (toxicity tests in cats), should be supplemented with data from other sources before interpretations concerning positive inotropic action can be made.

The informative value of the ATPase test, which measures the inhibitory effect of a heart glycoside on the Na^+, K^+-activated ATPase, is necessarily limited. According to several authors, it seems probable that the toxic, but not the inotropic effect is due to inhibition of the enzyme (see above). Substances with the desired pharmacological qualities (good inotropic effect with little toxicity) are especially easily overlooked[27], unless, as was done by Mendez et al.[13], the

17 T. Akera, S. I. Baskin, T. Tobin and T. M. Brody, Recent Adv. Stud. Card. Struct. Metab. *4*, 149 (1974).
18 C. Hegevary, Mol. Pharmac. *11*, 588 (1975).
19 R. Thomas, J. Boutagy and A. Gelbart, J. pharm. Sci. *63*, 1649 (1974).
20 R. V. Murthy, A. M. Kidwai and E. E. Daniel, J. Pharmac. exp. Ther. *188*, 575 (1974).
21 D. Ku, T. Akera, C. L. Pew and T. M. Brody, Naunyn-Schmiedebergs Arch. Pharmac. *285*, 185 (1974).
22 H. Lüllmann, T. Peters and K.-U. Seiler, Dt. med. Wschr. *96*, 1018 (1971).
23 H. Lüllmann and T. Peters, Eur. J. Pharmac. *14*, 204 (1971).
24 W. G. Sanborn and G. A. Langer, J. gen. Physiol. *56*, 191 (1970).
25 G. A. Langer and J. S. Frank, J. Cell Biol. *54*, 441 (1972).
26 W. G. Nayler, J. molec. Cell Cardiol. *5*, 101 (1973).
27 A. E. Ruoho, L. E. Hokin, R. J. Hemingway and S. M. Kupchan, Science *159*, 1354 (1968).

difference between inotropic and toxic doses is specifically determined on the isolated organ. Furthermore, the importance of the Hatcher test, which continues to belong to a first pharmacological screening of new potentially effective compounds, and which uses an enormous number of cats and expensively prepared compounds, must not be overestimated. New compounds are often only considered of medicinal interest and thus compared with one another[28], if they produce sufficient toxicity in vivo. Various publications show that conclusions based on the assumption still maintained by some, of an inseparable double effect of positive inotropy and toxicity are not reliable and may indeed even be misleading[29,30]. Wolff et al.[30] found with new compounds a high toxicity in the cat, but discovered later that cardiotonic properties, when tested on isolated atrium, were absent.

The test on isolated atrium of the guinea-pig, a classic model for the evaluation of heart glycosides, should not only be included in analyses of inotropic effects of such compounds, but should also be a regular feature in determining their therapeutic index. This can be done by increasing the concentration of the glycoside in the test bath, until the atria respond to the infusion with extrasystoles. One should be aware, however, that this test provides information about the inotropic effect and the toxicity of a substance exclusively, not about its antiarrhythmic properties. The use in therapy, however, of heart glycosides against insufficiency and arrhythmias[31] should not permit significant antiarrhythmical properties of a substance (such as distinctly marked antiarrhythmical along with weakly marked positive inotropic effects) to go unnoticed. This would unfortunately be the case, if the glycosides were exclisively tested on isolated atrium. The problems involved in the test methods used will become obvious in the following section about structure-activity relationships.

Structure-activity relationships

In order to determine the nature of the relationship between the structure of naturally occurring heart glycosides and their cardiotonic and -toxic effects, and thus possibly to learn something about the properties of their receptors, attempts have been made to determine what structural elements are absolutely indispensable for an inotropic response and what elements intensify the toxic effect[32]. Furthermore, it has been investigated as to how an existing effect could be altered by introducing new chemical groups into these compounds[33].

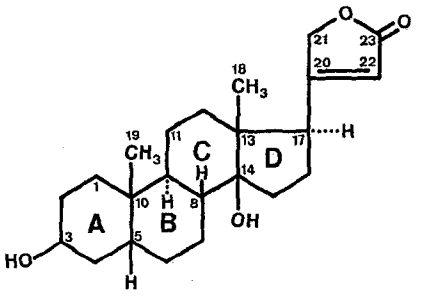
The concept of structure-activity relationships, as it appears today, is rather vague. 10–15 years ago the structural prerequisites for a cardioactive steroid seemed to be clear[32,34], but these dogmas have been questioned in recent investigations. Thus Kroneberg et al.[35] were able to produce steroidal mono- and bisguanylhydrazones (**XII**), whose pharmacological qualities coincided largely with those of cardioactive steroids of the cardenolide and bufadienolide type.

Doubtfulness of dogmas

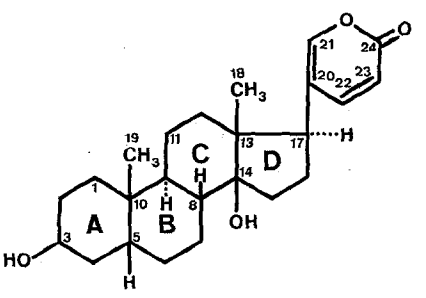
The dogmas, as described by Tamm[32], propose a 14β-hydroxy-steroid skeleton, in which, unless there are double bonds at C(4) or C(5), the rings A/B are in a cis, B/C in a trans and C/D in a cis configuration. Furthermore the steroids carry an unsaturated lactone as a 17β-side chain and a 3β-oriented oxygen function (OH-group or glycosidic linkage). Tamm assumed that further hydroxyl groups are of minor importance for a cardiac effect. He considered the distance between the lactone carbonyl and the oxygen function at C(3) to be the critical value for this effect[32,36]. This distance, exactly defined by the 2 electronegative poles and rigid steroid skeleton, should be closely connected with the properties of the physiological digitalis receptor. The sugar component in naturally occurring glycosides is of great importance with respect to kinetic behaviour of the substance in the organism[37,36]. But sugar-free compounds (genins) are also active, the genins with a 5-membered lactone as side chain (digitalis type), in contrast to those with a 6-membered lactone (scilla type), are less active (as determined by the toxicity test!) than the corresponding glycosides. Several synthetic modifications in the structure of heart glycosides disprove some of the above-mentioned obligatory prerequisites for a positive inotropic effect.

3β-OH. Saito et al.[38] have synthesized 3-desoxydigitoxigenin and found, on isolated frog heart (Straub's preparation), an activity comparable to that of digitoxigenin. Furthermore, Zürcher et al.[39] showed that in the ATPase test, this derivative loses only 50% activity. Witty et al.[40] have confirmed this result.

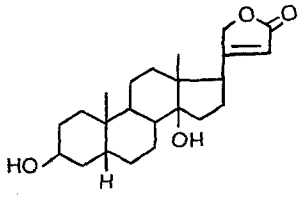
28 M. E. Wolff and W. Ho, J. pharm. Sci. *56*, 705 (1967).
29 A. H. El Masry, S. A. El Defrawy and O. Gisvold, J. pharm. Sci. *58*, 228 (1969).
30 M. E. Wolff, W. Ho and H.-H. Chang, J. med. Chem. *13*, 657 (1970).
31 J. E. Doherty and J. J. Kane, Drugs *6*, 182 (1973).
32 Ch. Tamm, in: Proc. 1st International Pharmac. Meeting, Stockholm, vol. 3, p. 11. Ed. W. Wilbrandt. Pergamon Press, Oxford 1963.
33 T. Shigei, H. Tsuru, Y. Saito and M. Okada, Experientia *29*, 449 (1973).
34 G. Kuschinsky and H. Lüllmann, in: Kurzes Lehrbuch der Pharmakologie, 4. Aufl., p. 51. Thieme, Stuttgart 1970.
35 G. Kroneberg, K. H. Meyer, E. Schraufstätter, S. Schütz and K. Stoepel, Naturwissenschaften *51*, 192 (1964).
36 K. R. H. Repke, Pharmazie *27*, 693 (1972).
37 R. J. Luchi and H. L. Conn, Prog. cardiovasc. Dis. *7*, 336 (1965).
38 Y. Saito, Y. Kanemasa and M. Okada, Chem. Pharm. Bull. *18*, 629 (1970).
39 W. Zürcher, E. Weiss-Berg and Ch. Tamm, Helv. chim. Acta *52*, 2449 (1969).
40 T. R. Witty, W. A. Remers and H. R. Besch, J. pharm. Sci. *64*, 1248 (1975).



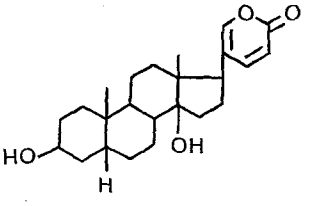
Cardenolide
(Digitalis Type)



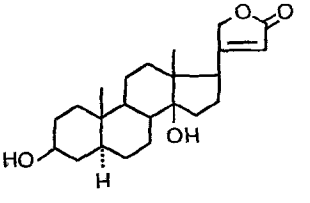
Bufadienolide
(Scilla/Bufo Type)



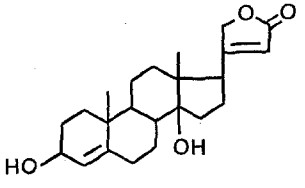
I Digitoxigenin



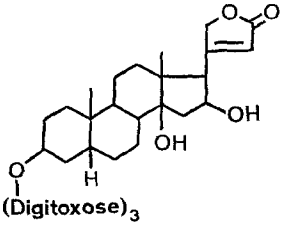
II Bufalin



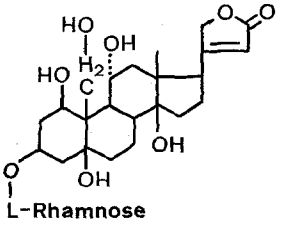
III Uzarigenin



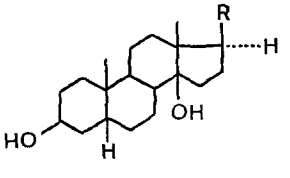
IV Canarigenin

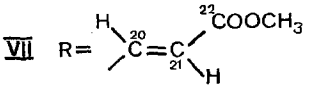


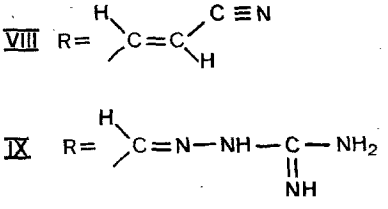
V Gitoxin

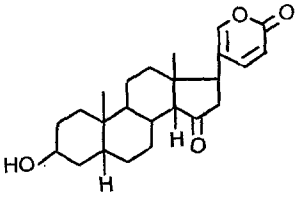


VI Ouabain

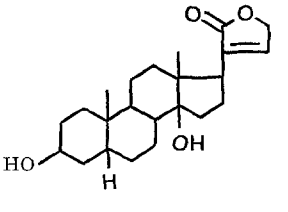




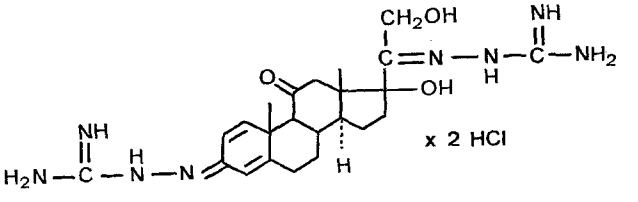




X 14β-Artebufogenin



XI



XII Prednison – 3.20 – bisguanylhydrazon x 2 HCl

They demonstrated, however, that hydrogenation of the lactone in 3-desoxy-digitoxigenin led to a far greater loss of activity (ATPase test) than that associated with the conversion of digitoxigenin to dihydro-digitoxigenin, a tendency observed in all tested 3-desoxy-compounds. On the other hand, a change in the configuration at C(3) of the cardenolide digitoxigenin (**I**), diminishes the effect in the ATPase test to about $^1/_{30}$ of that of digitoxigenin[39]. The corresponding bufadienolide 3α-bufalin still shows $^1/_7$ of the toxicity of bufalin (**II**) (Hatcher test)[41].

A/B-connection. The A/B-rings in natural glycosides generally show a cis configuration. A change of configuration at C(5) yields up to 10 times weaker products. The loss of activity (frog heart) in the cardenolides (digitalis range) seems to be less pronounced[42] than that in the bufadienolides (scilla range) – however Hatcher test![43].

Our knowledge of the influence of a double bond at C(4) → C(5) or C(5) → C(6) on inotropic and toxic effects is only rudimentary; scillaren A (Δ^4-bufadienolide) has a strong cardiotonic and cardiotoxic (Hatcher) effect, xysmalogenin (Δ^5-cardenolide) however has only weak cardiotoxic activity (Hatcher test only!)[44].

C/D-connection. Like the hydroxyl at C(3) the hydroxyl group at C(14) is not essential, but its substitution by 14β-H is accompanied by a considerable loss of activity: 14-desoxy-14βH-uzarigenin[45,46] and 14-desoxy-14βH-digitoxigenin[33] continued to show, on isolated frog heart, $^1/_3$ of the activity of uzarigenin (**III**) and about $^1/_{10}$ of the activity of digitoxigenin (**I**) respectively. The biological activity of 14β-artebufogenin (**X**) is also still present (Hatcher test however!)[47].

More important than the 14-hydroxyl group is the cis/β-configuration of the C/D-rings: 14α-compounds are ineffective throughout. 14-epi-digitoxigenin (ATPase test)[39] and 14α-artebufogenin (Hatcher test)[47] are inactive in contrast to the corresponding β-analogues. The rule that a trans-configuration of the C/D-rings is accompanied by a loss of activity[39,41], is without exception with all 'classic' heart glycosides. But synthetic prednison- (e.g. **XII**) and prednisolon-derivatives with largely similar effects to those of digitalis, have proved to be biologically active in spite of their 14α-H (isolated frog heart, isolated guinea-pig atrium, Langendorff heart, guinea-pig)[35].

Side chain. The lactone side chain is commonly regarded as the most essential functional group of cardiac glycosides. It is undisputed that this C(17)-side chain must be β-oriented – a change of configuration yields inactive compounds[32,36,38]. Deghenghi[48] was able to show that isomeric cardenolides (**XI**), differing from the natural ones only in the position of attachment of the butenolide ring to the steroidal 17β-position, are also active and even possess a more favorable therapeutic index than naturally occurring representatives. With this information, it was decided to investigate the influence of structural modifications at the butenolide ring on the pharmacological effect. Mendez at al.[13] noticed in experiments with the same substance that the difference between a minimal therapeutic and an arrhythmogenic or lethal dose, as well as the difference between an arrhythmogenic and a lethal dose, is more favorable than in glycosides with a normal lactone configuration.

Boutagy et al.[19,49], found in an extensive investigation on the role of the side chain in the effectiveness of cardenolides, that substitution of the C(17)-side chain in digitoxigenin (**I**), leading to the trans acrylic acid-methyl-ester (**VII**) and the trans-acrylonitrile (**VIII**), results in active compounds. In the Na^+, K^+-ATPase test as well as in improvement of the contractile force, both showed the same effectiveness as digitoxigenin (cf. also[50]). With a guanylhydrazoic group as side chain, the effectiveness was slightly reduced, but was still of comparable size. Going from the 17β-ester **VII** to the 17α-analogue, i.e. alteration of the configuration, also led to inactive compounds. The increase of the distance between the ester carbonyl in **VII** and the steroid nucleus by introducing a conjugated double bond (alltrans dien-system), was accompanied by an extensive loss of activity. The carbonyl group as such is not essential, as can be seen from the effectiveness of nitrile **VIII**. The distribution of the electron density in the side chain, however, seems to be important. From their results, Boutagy et al.[19,49], conclude that the lactone side chain does not constitute an indispensable structural feature (cf. also[51]) but that the structural element

$$\begin{matrix} & R'' & & \delta- \\ & | & & \\ -C= & CH- & C= & A \\ \delta+ & & | & \\ & & R' & \end{matrix}$$

in the C(17)β-side chain seems to be a prerequisite for cardiotonic steroids, A being a heteroatom (e.g. =O, ≡N), R′ usually as well and R″ H or an alkoxyl group. This structural element is lacking in ineffective saturated lactones.

41 K. Meyer, Planta med., suppl. 4, 1971.
42 N. Ishikawa, H. Tsurn, T. Shigei, T. Anjyo and M. Okada, Experientia *30*, 1308 (1974).
43 P. G. Marshall, in: Rodd's Chemistry of Carbon Compounds, 2nd ed., vol. II, part D, p. 360. Ed. S. Coffrey. Elsevier Publ. Co., Amsterdam, London, New York 1970.
44 J. G. Baumgarten, in: Die herzwirksamen Glykoside, p. 212. Thieme Verlag, Leipzig 1963.
45 M. Okada and Y. Saito, Chem. Pharm. Bull. *16*, 2223 (1968).
46 T. Shigei and S. Mineshita, Experientia *24*, 466 (1968).
47 M. S. Ragab, H. H. A. Linde and K. Meyer, Helv. chim. Acta *45*, 1794 (1962).
48 R. Deghenghi, in: Chemistry of Natural Products. 6. Internat. Symp. Mexico City 1969, p.153. Butterworths London 1970.
49 J. Boutagy, A. Gelbart and R. Thomas, Aust. J. pharm. Sci. *2*, 41 (1973).
50 W. Eberlein, J. Heider and H. Machleidt, Chem. Ber. *107*, 1275 (1974).
51 H.-J. Portius and K. Repke, Arzneimittel-Forsch. *14*, 1073 (1964).

Eberlein et al.[50], who replaced the butenolide ring in 14β-hydroxy-C/D-cis-steroid-glycosides and -aglycones by open side chains, attained similar results. Acrylic acid-esters and -nitriles showed typical cardioglycosidic effects in vivo (isolated guinea-pig atrium, dog, cat) and in vitro (Na^+, K^+-ATPase inhibition at human erythrocytes).

The influence of additional structural modifications

When studying structure-activity relationships of cardiac glycosides, attempts have also been made at introducing new functional groups or atoms into different positions of the steroid nucleus in order to facilitate an uncoupling of cardiotonic from cardiotoxic effects.

Halogens. Eberlein et al.[52] have substituted the vinylic hydrogen at C(22) of the lactone with halogen, alkyl and alkoxyl groups. By introducing fluorine into the butenolide ring of digoxin, they achieved an increase of contraction at the isolated guinea-pig atrium, while a strong decrease was noticed after introducing a methoxyl group. They consider a conjugated 4-centre-π-system, with the negative charge centre at its free end, as a minimal prerequisite for cardiotonic activity[50].

Chlorine was introduced into different positions of the steroid nucleus. In doing so, active compounds could in some cases be obtained. None of them, however, surpassed the natural glycosides in their pharmacological qualities. Meyer[53] was able to attach a chlorine atom at 15α-position of bufalin (**II**), but this 'resibufogenin-hydrochlorid' showed no toxicity. Stache et al.[54] substituted the hydrogen at C(4) by chlorine in canarigenin (**IV**), 14, 15β-epoxy-canarigenin and 14-desoxy-14αH-canarigenin. When tested for positive inotropic activity on isolated left atrium of guinea-pigs, 4-chloro-canarigenin was about as active as canarigenin itself while, as expected, 14, 15β-epoxy-canarigenin was devoid of any activity.

C(14)C(15). Relationships between the structure at C(14) and C(15) and the biological activity are still obscure and somewhat contradictory. The obligatory β-position of a hydroxyl at C(14) has been referred to above. Experiments by Shigei and by Okada et al.[33, 46, 55, 56] showed that the introduction of an oxygen function at C(15) in digitoxigenin, always resulted in a decrease of pharmacological activity, when tested on isolated frog heart (Straub): digitoxigenin (relative activity 1.0) > 15β-hydroxy-digitoxigenin (0.1) > 15-oxo-digitoxigenin (0.02) > 15α-hydroxy-digitoxigenin (inactive). In the series of 14-desoxy-14βH-digitoxigenin, the activity also decreases, but the 14-desoxy-14βH-digitoxigenin itself has a relative activity of only 0.04-0.15, whereas the 15-oxo-derivative still retains 10-30% of the activity of digitoxigenin. This last result, however, is in contrast with the results of Henderson and Chen (Hatcher test[57]), who found 14βH-15-oxo-digitoxigenin to be ineffective[41]. In contrast to the Henderson and Chen result is the finding that the relevant bufadienolide, 14β-artebufogenin (**X**), is also active (Hatcher test)[41]. Both in the series of digitoxigenin and that of 14-desoxy-14βH-digitoxigenin, the 15α-OH-compound is ineffective. To conclude from this, however, that 15α-hydroxy-compounds of cardiac glycosides are generally inactive would be premature. In the series of 14-desoxy-14β-chloro-digitoxigenin derivatives, the 15β-hydroxy- and 15-oxo-derivatives are both without effect, whereas the 15α-hydroxy-analogue still shows 10-30% of the effect of digitoxigenin[33].

14β, 15β-epoxides are in general less effective than the respective 14β-OH-compounds[58]. Their activity is, however, supposed to be frequently linked to a better therapeutic index. (Personal communication from Prof. K. Meyer, Basle University.) A careful evaluation of the therapeutic index of such epoxides would therefore be desirable and of great importance, as here might be found a means to reduce risks in heart glycoside therapy.

C(16). In the cardenolide series, gitoxigenin derivatives have attracted increasing interest in recent years. Kovařiková et al.[59] concluded from their experiments with gitoxigenin and its 16-epimer (electrically stimulated cat papillary muscle) that the 16-hydroxyl group, as such, causes an unfavorable effect. On the other hand, Repke[36] found for the glycoside 16-epi-gitoxin at maximally inotropic concentrations, a lower frequency of arrhythmias than for gitoxin (**V**) or ouabain (**VI**) (isolated guinea-pig atrium). Furthermore 16-epi-gitoxin showed a wider dosage range than gitoxin or ouabain between minimal and maximal inotropic effect[60]. A partial separation – at least for the guinea-pig heart – of therapeutic and toxic effects seems to have been achieved with 16-epi-gitoxin and, according to Haustein et al.[61], with 16-acetal-16-epi-gitoxin. Both compounds have a stronger effect on the ventricular muscle and a weaker one on the atrium and on the Purkinje system, the latter being considered to be the source of most toxic effects of digitalis[62].

52 W. Eberlein, J. Nickl, J. Heider, G. Dahms and H. Machleidt, Chem. Ber. *105*, 3686 (1972).
53 K. Meyer, Helv. chim. Acta *35*, 2444 (1952).
54 U. Stache, K. Radscheit, W. Fritsch and W. Haede, Liebigs Ann. Chem. *1974*, 608.
55 T. Shigei, M. Katori, H. Murase and S. Imai, Experientia *20*, 572 (1964).
56 M. Okada and Y. Saito, Chem. Pharm. Bull. *15*, 352 (1967).
57 F. G. Henderson and K. K. Chen, J. med. Chem. *8*, 577 (1965).
58 B. K. Naidoo, T. R. Witty, W. A. Remers and H. R. Besch, J. pharm. Sci. *63*, 1391 (1974).
59 A. Kovařiková, H. Kolárová and J. Pitra, Experientia *20*, 263 (1964).
60 K.-O. Haustein, F. Markwardt and K. R. H. Repke, Eur. J. Pharmac. *10*, 1 (1970).
61 K.-O. Haustein and J. Hauptmann, Pharmacology *11*, 129 (1974).
62 M. Vasalle, J. Karis and B. F. Hoffman, Am. J. Physiol. *203*, 433 (1962).

N-analogues. Recently, various N-analogues of cardioactive cardenolides with promising pharmacological properties have been developed[41]. The compounds concerned are, e.g. 3α- and 3β-amino-3-desoxy-digitoxigenin, 3-amino-3-desoxy-derivatives of uzarigenin, oleandrigenin, gitoxigenin and digoxigenin prepared by Meyer et al.[63,64]. The same new class of compounds has been treated in several publications and has been the object of patent applications[65,66]; its therapeutic applicability is still being evaluated.

In conclusion, of the dogmas concerning structure-activity relationships as postulated by Tamm[32] and others[34,67] only those touching the 17β-configuration of the side chain and a cis configuration of the C/D-rings (i.e. 14β-H or some other 14β-substituent for possible exceptions[35,65]), are still valid. All other structural features, such as are found e.g. in digitoxigenin, do not necessarily involve a complete loss of effectiveness when modified. But none of the various structural modifications have, up to now, resulted in a compound with either pharmacological properties superior to classical cardiac glycosides or with a better therapeutic index. The hope, however, that further partially synthetic modifications will realize this goal is certainly justified.

63 L. Sawlewicz, E. Weiss, H. H. A. Linde and K. Meyer, Helv. chim. Acta *55*, 2452 (1972).
64 E. Hauser, U. Boffo, L. Meister, L. Sawlewicz, H. H. A. Linde and K. Meyer, Helv. chim. Acta *56*, 2782 (1973).
65 U. Stache, W. Haede, W. Fritsch, K. Radscheit and E. Lindner, DOS 2013032 (1971), CA 76, 14816 b (1972).
66 K. Meyer, DOS 2137047 (1972), CA 76, 141218, CA 84, 59879.
67 R. Mutschler, in: Arzneimittelwirkungen, 1. Aufl., p. 236. Wissenschaftl. Verlagsgesellschaft, Stuttgart 1970.

SPECIALIA

The editors do not hold themselves responsible for the opinions expressed in the authors' brief reports. – Les auteurs sont seuls responsables des opinions exprimées dans ces brèves communications. – Für die Kurzmitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – Ответственность за короткие сообщения несёт исключительно автор. – Solo los autores son responsables de las opiniones expresadas en estas comunicationes breves.

On the cyclization of farnesic acid[1]

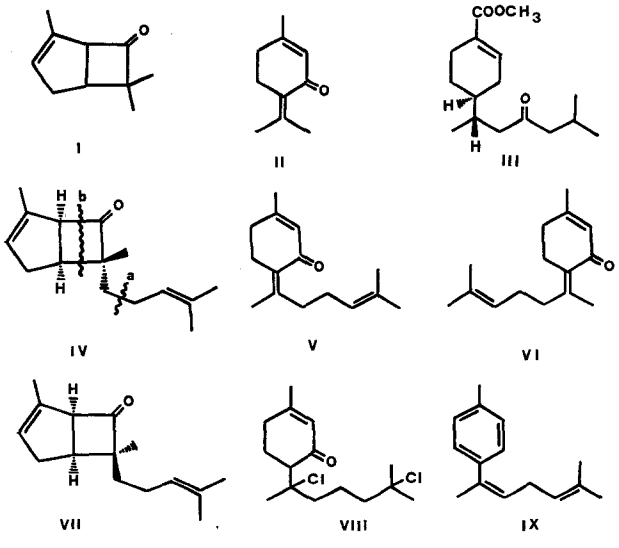
A. Corbella, P. Gariboldi, Myrna Gil-Quintero, G. Jommi and J. St. Pyrek[2]

Laboratorio di Chimica Organica, Facoltà di Scienze, Università degli Studi, via Saldini 50, I-20133 Milano (Italy),

9 September 1976

Summary. Acetic anhydride treatment of farnesic acids leads to the expected cyclohexenones (**V** and **VI**) and to the alternative formation of the [3. 2. 0]-bicycloheptenones (**IV** or **VII**) depending on the geometry of the central double bond of the acid.

The reaction of geranic acid with acetic anhydride and sodium acetate has been reported[3] to give mainly 2 products, filifolone (**I**) and piperitenone (**II**). In the search of compounds with potential hormonal activity on insects, we have done an analogous reaction with the farnesic acids on the basis of the following arguments. The esters of farnesic acids[4] display JH activity on a number of insects, but it is not known whether the compound is active as such or whether a metabolite is the true hormone.



Furthermore, some of the possible cyclization products of the farnesic acids resemble the molecule of juvabione (**III**), so that it would be of some interest to test their activity. Again, filifolone itself has been shown to display juvenoid activity on some insects[5].

Treatment of a mixture of 2-trans,6-trans- and 2-cis,6-trans-farnesic acids with acetic anhydride and sodium acetate in the conditions described by Beereboom[3] gave 3 main products which have been separated[6] by repeated silica-gel columns and identified as **IV**, **V** and **VI**.

Assignment of the structure **IV** to the compound of higher volatility is based mainly on its physicochemical data and on considerations of the reaction mechanism. The IR-spectrum has an absorption band at 1770 cm^{-1} due

1 This work has received financial support from Consiglio Nazionale delle Ricerche.

2 Present address: Inst. Org. Chem. PAN, Kasprzaka 44, Warszawa, Poland.
3 J. J. Beereboom, J. org. Chem. *30*, 4230 (1965).
4 See for references: K. Šlama, M. Romaňuk and F. Šorm, Insect Hormones and Bioanalogues. Springer-Verlag, Wien, New York 1974.
5 Unpublished results.
6 Conversion of farnesic acid is about 60%, the remaining being recovered unchanged. The yields of **IV**, **V** and **VI**, as a mean of 3 runs, are 40, 32 and 28% respectively. Column chromatography on silica-gel (absorbent:substance 30:1) eluting with hexane:AcOEt 95:5, easily separates **IV** from **V** and **VI**; these can be obtained in pure form only by a second chromatography (absorbent : substance 120 : 1) eluting with hexane : AcOEt 9 : 1.