A New Approach to the Design of Novel Inhibitors of Na^+,K^+-ATPase: 17α-Substituted Seco-D 5β-Androstane as Cassaine Analogues

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Received February 18, 1998

A new three-dimensional model for the relative binding mode of cassaine **1** and digitoxigenin **2** at the digitalis receptor site is proposed on the basis of the structural and conformational similarities among **1**, **2** and its 14,15-seco analogues **3** and **4**. Accordingly, the speculation that also 17α-substituted derivatives of the digitalis 5β,14β-androstane skeleton could efficiently bind to the Na^+,K^+-ATPase receptor is put forward and verified through the synthesis of some related compounds. The binding affinity shown by 2-(*N*,*N*-dimethylamino)ethyl 3β,14-dihydroxy-5β,14β-androstane-17α-acrylate **6** (IC_{50} = 5.89 μM) and, much more significantly, by the corresponding 14,15-seco-14-oxo derivative **9** (IC_{50} = 0.12 μM) substantiates the new hypothesis and opens new prospects to the design of novel inhibitors of Na^+,K^+-ATPase as potential positive inotropic compounds.

Introduction

The search for new Na^+,K^+-ATPase inhibitors as positive inotropic compounds with an improved therapeutic ratio with respect to the natural cardiotonic steroids has been a challenge since the 1960s but represents a still unachieved goal in medicinal chemistry. Some authors suggest that the possibility of separating toxic from positive inotropic effects may reside in compounds able to discriminate among the different isoforms of the target enzyme.[1a−e] In any case, the question whether it is theoretically and/or practically possible to improve the therapeutic index of digitalis inotropic drugs has not been answered yet. The limited structural diversity of the skeletons taken as a basis for chemical modification is probably one reason for the lack of success, but on the other hand, the design of novel inhibitors of Na^+,K^+-ATPase starting from different steroidal skeletons, or even nonsteroidal ones, is usually faced with poor affinity and selectivity for the target receptor.[1c,2] As part of our work in this field, we report some interesting molecular modeling results and put forward new suggestions on the structure−activity relationships of digitalis-like compounds. This may contribute to stimulation of further research in unexplored chemical classes and hopefully bring about the synthesis of structurally diverse lead compounds. The synthesis and biological evaluation of novel inhibitors in the 14,15-seco-5β-androstane series will be also reported.

The Digitalis Pharmacophore. The problem of identifying the essential structural requirements for inhibiting Na^+,K^+-ATPase, i.e., the "digitalis pharmacophore", has been the subject of several studies during the past decades,[1a,b] but its definition is far from being clearly accomplished. The specificity of the biological action of digitalis drugs has been always ascribed to the peculiar structural features of these compounds, as the result of a unique three-dimensional spatial disposition of the substituents.[3] However, it is well-known that none of the polar substituents is indispensable for activity and that steroidal aglycons with A/B trans and/or C/D trans geometry can likewise serve as basic structures for the synthesis of compounds which are full inhibitors, although at much higher concentrations.[1a−c,2,4] The only residual digitalis feature that seems to be essential for high affinity, beyond the important hydrophobic interaction of the steroidal skeleton,[5] is the 17β stereochemistry, but, as will be demonstrated below, the absolute configuration at C17 can also be altered, retaining considerable activity.

Cassaine Analogues and Seco-D Digitalis-like Compounds. Among the nonsteroidal inhibitors of Na^+,K^+-ATPase, the *Erythrophleum* alkaloid cassaine **1** has been recognized for many years to possess a pharmacological action similar to that produced by digitalis glycosides, also with respect to the mechanistic model of inhibition.[1e,6] Its main chemical features are (a) a lipophilic perhydrophenanthrene nucleus; (b) the presence of an α,β-unsaturated ester moiety, a feature common to the 17β-substituents of classic cardioactive steroids; (c) a basic residue which is essential for its activity.[6] The importance of an ionic interaction in the region of the 17-substituent of Na^+,K^+-ATPase inhibitors has been demonstrated by SAR studies also for digitalis-like compounds.[1a,5,7,8]

In 1991, R. W. Baker et al.[9] reported the synthesis of a series of cassaine analogues and demonstrated a significant contribution to the binding energy displayed by a hydrophobic interaction in the α region, approximately corresponding to the D-ring of digitalis compounds (C15−C16). The superimposition between the cassaine and digitoxigenin skeletons proposed by the Australian authors is based on the traditional matching

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S0022-2623(98)00108-3 CCC: $15.00
Published on Web 07/11/1998

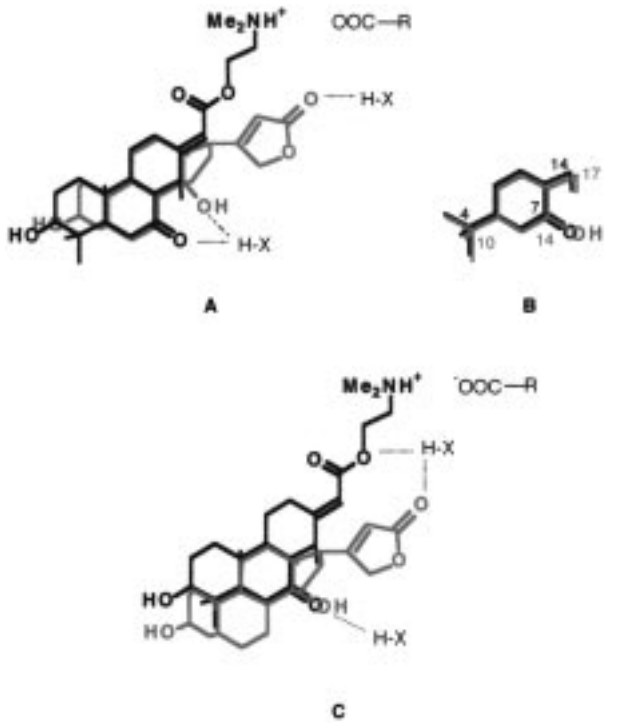
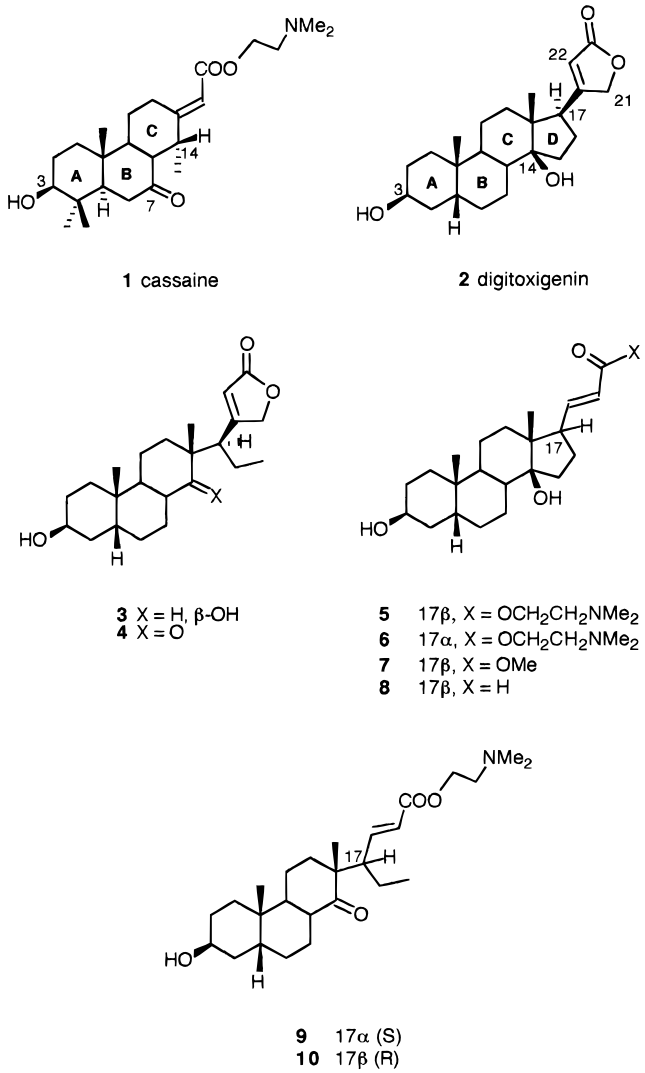


Figure 1. Schematic superposition of cassaine **1** (blue) and digitoxigenin **2** (red). A: classic matching of perhydrophenanthrene nucleus. B: common fragment. C: proposed model.

of the A, B, and C rings (Figure 1A) but is rather unsatisfactory with regard to the α,*β*-unsaturated ester region.[9] This alignment is substantially retained in Höltje's pseudo-receptor model,[10] where it is also hypothesized that the amino group of cassaine could interact with the same H-donor on the receptor that is possibly involved in the binding of 16*β*-OCOR derivatives.[11]

In this context, the role of the 7-oxo group of cassaine should be that of an H-bond acceptor, analogous to the 14-hydroxy or the 14,15-oxido groups in natural cardenolides. In both cases, the H-donor counterpart on the enzyme might be effectively located between the two bond directions C7−O and C14−O (Figure 1A).[10]

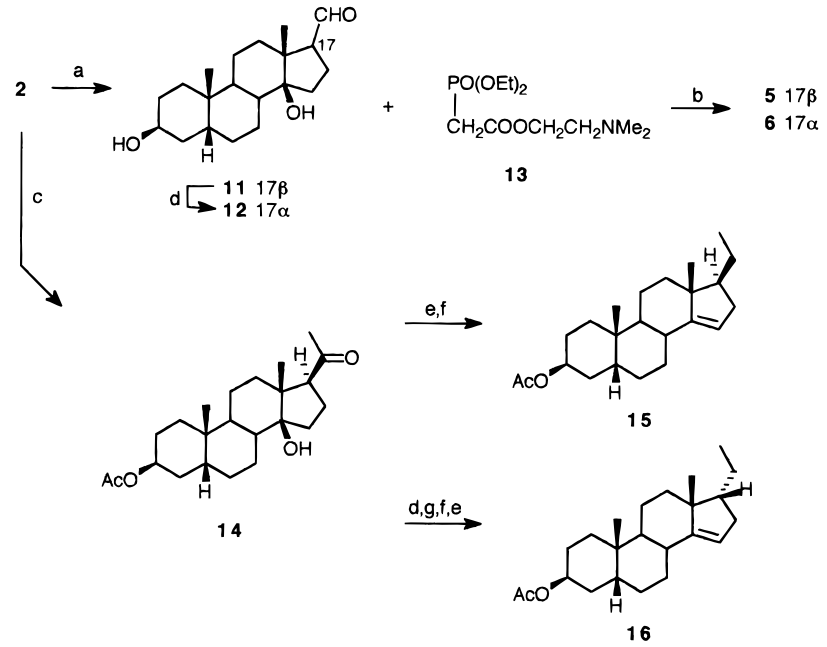
Transfer of this most interesting piece of information, i.e., the α-hydrophobic interaction, into the seco-D digitoxigenin series recently reported by some of us[12] resulted in a significant increase in potency for compounds **3** and **4**, characterized by the presence of the C15−C16 ethyl group, compared to the more hydrophilic 15-oxidized derivatives.[13] This rationale was not reported at that time due to the immaturity of the model but strengthened our feeling about the close structural correspondence between cassaine C7/C14 and 14-oxo-14,15-secodigitoxigenin C14/C17 functional groups, respectively (Figure 1B). Moreover, we showed that the ketone function at position C14 of the seco-D derivatives is a good substitute for the 14-OH as an H-bond acceptor in the interaction with the receptor. Therefore, we considered that this parallelism could be better described by a different relative spatial alignment of the two skeletons and postulated that ring B of cassaine corresponds to ring C of digitoxigenin, although the other rings superpose only partially. As a result of this translation that makes the two oxygen-bearing carbons, C7 of cassaine and C14 of digitoxigenin, coincide (Figure 1C), the 14α-methyl group of the alkaloid is located very close to the C16 methylene of the steroid, but the most striking peculiarity of this novel superposition is that the unsaturated ester of cassaine occupies a very different position in space, namely it stretches out in the 17α region of digitoxigenin (Figure 1C). Notwithstanding the reported very low activity, if any, of 17α derivatives,[2,14a] and the expert belief that the *β* stereochemistry at C17 is a fundamental feature in digitalis-like compounds, the 17α-epimer of digitoxigenin showed, in our binding test, an IC_{50} value (5 *μ*M) which indicated an enhanced interaction with the receptor, compared to the 17-unsubstituted steroidal skeleton (5*β*,14*β*-androstane-3,14-diol, IC_{50} > 100 *μ*M).[5] We thus hypothesized that both a hydrogen bond and an ionic interaction could be formed also by the 17α-dimethylaminoethyl ester moiety of cassaine on a seco-D digitalis skeleton and synthesized the 17α derivative **9**. For comparison purposes, we synthesized also the 17*β* isomer **10** and the corresponding 17*β*- and 17α-acrylate digitalis analogues (compounds **5** and **6**, respectively).



Synthesis

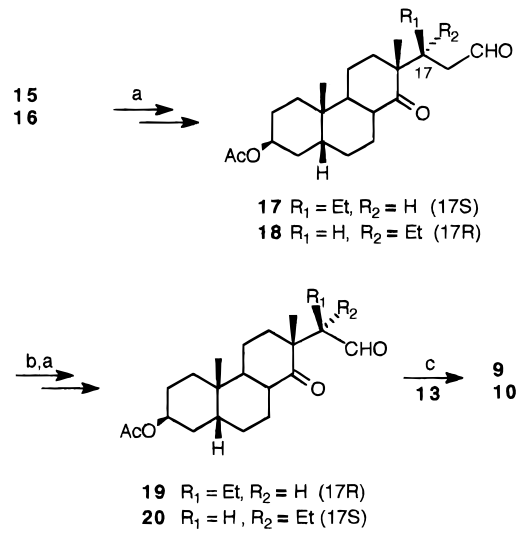
The synthesis of compounds **3**, **4**, **7**, and **8** has been already reported.[2,12,15] The target compounds **5**[16] and **6** were straightforwardly obtained from 3*β*,14*β*-dihydroxyetianaldehyde[17] **11** and its 17α-epimer **12**,[14b]

Scheme 1[a]



[a] Reagents and conditions: (a) ref 17; (b) NaH, THF, room temperature; (c) ref 18; (d) KOH/MeOH; (e) $SOCl_2$, pyridine, 0 °C to room temperature; (f) *p*-toluensulfonhydrazide, AcOH, room temperature; then $NaBH_3CN$, ZnI_2 cat., MeOH, Δ; (g) Ac_2O, pyridine.

Scheme 2[a]



[a] Reagents and conditions: (a) O_3, CH_2Cl_2, −78 °C; then Zn, AcOH, room temperature; (b) Ac_2O, TEA, CH_2Cl_2, room temperature; (c) NaH, THF, room temperature; then 5% HCl/dioxane.

respectively, by a Horner−Emmons reaction with phosphonate **13** (Scheme 1). Compounds **9** (single *E*-isomer) and **10** (obtained as a 4:1 mixture of *E*/*Z*-isomers) were synthesized similarly from 14,15-seco 17-aldehydes **19** and **20**, respectively, which were in turn obtained through a multistep sequence starting from 3*β*-acetoxy-14-hydroxy-5*β*,14*β*-pregnan-20-one[18] **14** (Schemes 1 and 2). Briefly, compound **14** was first dehydrated with $SOCl_2$ in pyridine to the 14,15-ene, and then the methyl ketone was reduced to 17*β*-ethyl group via tosylhydrazone. Oxidative cleavage with ozone of the 14,15-ene derivative **15** gave keto aldehyde **17**. The corresponding 15-enolacetate was then ozonized again to give the desired aldehyde **19** (Scheme 2). The seco-D 5*β*-androstane-17*β*-carbaldehyde **20** was obtained in a quite similar fashion starting from the 17α-epimer of **14**,[19] through a slightly different step sequence (Scheme 1). It is well-known that the 17*β* configuration is thermodynamically more stable in C/D-trans or 14,15-ene steroid derivatives, while 17α-isomers are more stable in the C/D-cis series.[14b,19] To avoid the epimerization at C17, which actually occurred during the formation of the 3*β*-acetoxy-5*β*,17α-pregn-14-en-20-one tosylhydrazone, the reduction of the 17α-methyl ketone was carried out before dehydration to the 14,15-ene derivative **16** (Scheme 1).

Results and Discussion

The compounds were evaluated for their ability to inhibit the specific [^{3}H]ouabain binding to dog kidney Na^+,K^+-ATPase. The corresponding IC_{50} values are reported in Table 1 (column 2). To rationalize the consequences of C17 epimerization and D ring breaking on structure activity relationships, we carried out systematic conformational analyses on digitoxigenin **2**, its seco analogues **3** and **4**, and the 17-acrylate derivatives **5**−**10**. Rotation about the C13−C17 and C17−C20 bonds was accomplished using the MM2 dihedral driver option. All conformations representing the local minima found were successively fully minimized within both the MM2(91) force field and AM1 semiempirical method. Relative energy values and torsional angles for the conformations thus obtained are reported in Table 1.

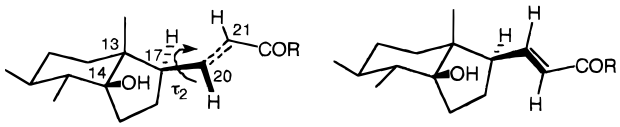
It is interesting to observe that the introduction of a dimethylamino group in acrylate derivatives results in a marked increase of affinity in the case of **5** with respect to methyl acrylate **7** or bulkier esters.[14a] The very high affinity of **5** (0.03 μM) confirms the importance of an ionic binding point besides the dipolar interaction of the α,*β*-unsaturated ester. Also in the case of the 17α-epimer **6**, notwithstanding its low IC_{50} value, the introduction of a basic residue increases affinity in comparison to the reported inactivity of methyl 17α-acrylate analogue (0.003 relative potency vs. digitoxigenin).[14a] It is thus possible to imagine that both

Table 1. Binding Affinities, Geometry, and Relative Energy of Minimized Conformations for Digitoxigenin, Seco-D Derivatives, and Cassaine-like Analogues

compd	binding[a] IC_{50} (μM)	conform[b]	τ_1[c]	τ_2	ΔE (kcal mol^{-1}) MM2	AM1
1	*d*		−175	130		
2	0.08	X-ray[e]	−104	76		
		A	−98	−114	0	0
		B	−97	72	0.34	0.30
3	0.13	**A**	−86	−89	1.85	1.94
		B	−87	75	1.42	2.12
		C	47	78	0	0
		D	42	−118	0.14	2.18
4	0.20	**A**	−82	−98	1.11	0.75
		B	−81	71	1.96	0.84
		C	60	79	1.42	0
		D	45	−127	0	2.40
5	0.03	**A**	−88	−125	0	0
		B	−99	81	0.76	2.33
6	5.89	**E**	159	113	0	0
		F	162	−150	0.46	0.1
7	0.35	**A**	−88	−125	0	0
		B	−98	83	0.95	1.88
8	0.06	**A**	−89	−126	0	0
		B	−98	82	1.03	2.85
9	0.12	**E**	−170	130	0	0
		F	−166	−88	0.90	1.58
		C	55	110	0.50	0.9
		D	68	−83	2.01	3.10
10	0.25[f]	**A**	−75	−128	1.14	0.20
		B	−83	83	0.90	2.26
		C	59	113	2.45	0.91
		D	72	−94	0	0

[a] Determined in a competitive binding assay employing [^{3}H]ouabain as displaced ligand. IC_{50} values are means of two or three independent experiments in duplicate. [b] In 17β isomers, A is the digitoxigenin-like conformation, with the α,β-unsaturated carbonyl in the s-trans conformation. In 17α isomers, E is the cassaine-like conformation, with the α,β-unsaturated carbonyl in the s-cis arrangement. [c] τ_1, dihedral angle C14−C13−C17−C20 (deg); τ_2, dihedral angle C13−C17−C20−C22 or −C21 in 17-acrylate derivatives; AM1 geometry. [d] For comparison, the relative potency of cassaine vs digitoxigenin, as determined from inhibition assays on different enzymes, is ca. 0.1. References 5a, 6. [e] Reference 26. [f] Data obtained from a mixture of *E*/*Z* (4:1) isomers.

Chart 1. Low-Energy Conformations for 17β-Acrylate Derivatives

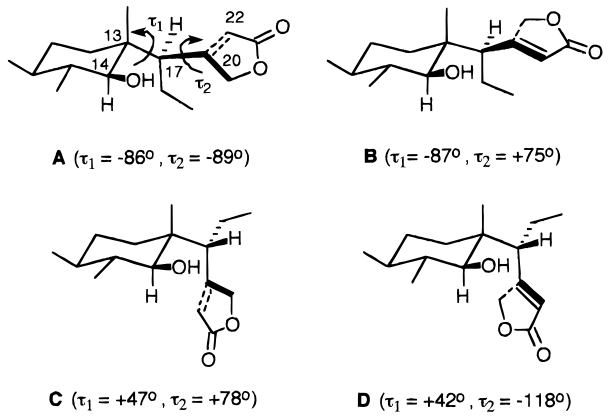


A (τ_2 = -125°) **B** (τ_2 = +82°)

17α and 17β basic chains in extended conformations can reach the same anionic site in the receptor.[20]

The minimum energy conformation of compounds **5**, **7**, and **8** (Chart 1, **A**), where the C20−H bond is antiperiplanar to C17−H, is significantly more stable than the other one (Chart 1, **B**), as confirmed also by the value of the vicinal coupling constant *J*(H-17/H-20) = 10.5 Hz in the ^{1}H NMR spectrum, which stands for an almost exclusive antiperiplanar arrangement of coupled atoms.[4] Conversely, the energy difference between the two orientations of the butenolide ring is very low in the digitoxigenin case (0.34 kcal mol^{-1}). Thus, the high affinity of acrylaldehyde **8**, equipotent to **2**, suggests that the putative binding conformation of digitoxigenin is **A** (Table 1), where the double bond C20−C22 eclipses the C17−H bond, as previously hypothesized.[21,22]

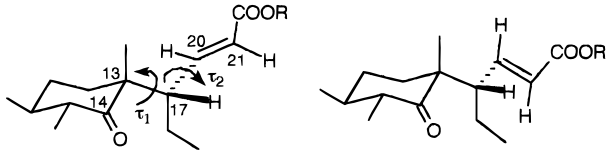
Chart 2. Low-Energy Conformations for Compound **3**



A (τ_1 = -86°, τ_2 = -89°) **B** (τ_1 = -87°, τ_2 = +75°)

C (τ_1 = +47°, τ_2 = +78°) **D** (τ_1 = +42°, τ_2 = -118°)

Chart 3. Low-Energy Conformations for Compound **9**



E (τ_1 = -170°, τ_2 = 130°) **F** (τ_1 = -166°, τ_2 = -88°)

One of the traditional approaches in rational drug design to maximize affinity levels consists of the synthesis of conformationally constrained derivatives. The consequent reduction in conformational entropy results in an increase of the apparent affinity provided that the "rigid" structure corresponds the receptor bound conformation of the unconstrained analogue. In the case of 14,15-seco compounds[12] we made an opposite process by the C14−C15 bond rupture, i.e., we increased the conformational freedom, with the aim of studying the consequences on conformational behavior and affinity levels. Four local minima were identified by a conformational search (**A**−**D** in Chart 2). The minimum MM2 energy conformations of **3** and **4** (Table 1, **C** or **D**, respectively), where the butenolide points downward (Chart 2, **C** or **D**), are not likely to be the "active" ones. The best superposition with the digitoxigenin template is obtained with conformation **A**, which is less stable by 1−2 kcal mol^{-1} higher internal energy (MM2 values, Table 1). On this basis, a considerable reduction in binding affinity should be anticipated for seco compounds. The relatively high affinities retained by **3**, **4**, and **10** seem to indicate that the increase in conformational freedom[23] is counterbalanced by a stronger interaction with the receptor of the C15−C16 ethyl group, possibly through van der Waals forces, as in the case of the cassaine analogues cited above.[9] On the contrary, the minimum energy conformation for **9** (Chart 3, **E**) is the one that best reproduces cassaine geometry (τ_2 = 130° in both cases, Figure 2). Therefore, the high activity of compound **9** (50-fold affinity increase vs **6**) is in line with the proposed alignment of cassaine and

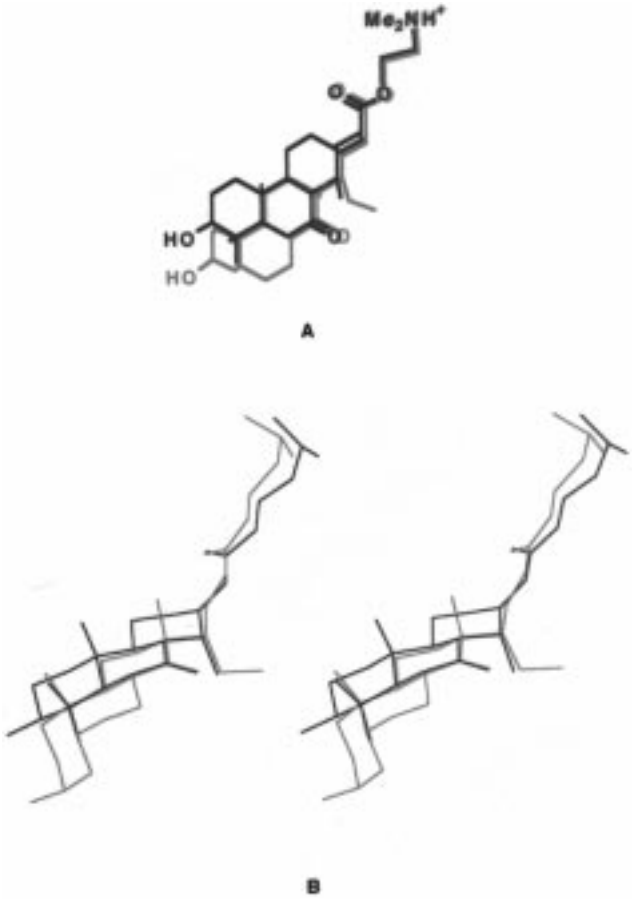


Figure 2. A: Schematic superposition of cassaine **1** (blue) and seco-D 17α-acrylate **9** (red). B: Stereoview of the same superposition.

digitoxigenin (Figure 1C) and the good superimposition between **9** and cassaine (Figure 2). This indicates that the putative binding conformation of the 17α-epimer is much more favored in the seco-D derivative **9** then in the digitalis-like derivative **6**, where the D ring is intact. In summary, the increase of conformational freedom lowers the affinity of the 17β derivative, whereas the affinity of the 17α-epimer is increased, due to the more effective arrangement of the pharmacophoric points in the minimum energy conformation. This compensates for the loss of the 17β-dipolar interaction and explains the similar affinity value obtained for the two epimers.

Conclusion

On the basis of the structural and stereochemical parallels among cassaine, digitoxigenin, and 14,15-secodigitoxigenin analogues, we have drawn a new model for the relative alignment of cassaine at the digitalis receptor. As a consequence, 17α-dimethylaminoethyl-substituted analogues of digitoxigenin were predicted to be potential inhibitors of Na^+,K^+-ATPase. Some affinity for the digitalis receptor was effectively shown by the dimethylaminoethyl 5β,14β-androstane-17α-acrilate **6**, while the high affinity of the corresponding 14,15-seco derivative **9** (IC_{50} = 0.12 μM) has confirmed the hypothesis that seco-D digitoxigenin analogues can be considered cassaine mimics. The high affinity displayed by **9** has been rationalized on the basis of the conformational preference for a cassaine-like conformation. These results demonstrate that it is possible to design Na^+,K^+-ATPase inhibitors without being constrained by the stereochemistry of the classic digitalis skeleton in the D-ring region. Further work is in progress to synthesize novel 17α derivatives by substituting the unsaturated ester function with more effective dipolar groups[27] and to study the pharmacological profile of these novel structural templates.

Experimental Section

General. Melting points were determined on a Büchi 535 melting point capillary apparatus and are uncorrected. Elemental analyses were carried out by Redox, Cologno Monzese (Milan, Italy), and the obtained analytical results were within ±0.4% of the theoretical values. ^{1}H NMR (^{13}C) spectra were recorded on a Bruker AC300 instrument operating at 300.13 (75.48) MHz in $CDCl_3$ or CD_3OD solutions. Chemical shifts are reported as parts per million (δ) relative to tetramethylsilane (TMS). The residual signals of the solvents were used as internal reference and set at δ 7.27 ($CHCl_3$) and 3.31 (CD_2-HOD) for proton chemical shifts (δ_C 77.0 and 49.0 for ^{13}C, respectively). Coupling constants (J) and width at half-height ($W_{h/2}$) values are reported in hertz. ^{13}C signals were classified as to multiplicity with the DEPT technique. IR spectra were recorded on a Perkin-Elmer 1710 instrument. Mass spectra were taken in electron impact mode (EI, 70 eV) on a Finnigan-Mat Incos 50 instrument, using the Direct Exposure Probe (DEP) technique. Reactions were monitored by thin-layer chromatography (TLC) on silica gel plates with fluorescent indicator (Merck). Flash column chromatography (FCC) was performed on silica gel (Merck, 40−63 mesh). Solutions were dried using anhydrous Na_2SO_4 and evaporated under reduced pressure. Tetrahydrofuran (THF) was dried by distillation from sodium metal with benzophenone as an indicator.

Molecular Modeling. All computations were performed on a Macintosh Performa 6400/200 running MacMimic/MM2-(91) (v. 3.0, Instar Software AB, Ideon Research Park, S-223 70 Lund, Sweden) and MacSpartan/Mopac/AM1 (v. 1.1, Wavefunction Inc., 18401 Von Karman Ave., Suite 370, Irvine, CA 92715) software. Structures were minimized in vacuo. Dihedral angles C15−C14−O−H in compounds **2**, **3**, **6**, **7**, and **8** and C13−C17−C16−C15 in derivatives **3**, **4**, **9**, and **10** were set antiperiplanar in the starting geometry. Also the dimethylaminoethyl fragment was kept in the extended conformation in all instances.

Biological Test. Binding affinity for dog kidney Na^+,K^+-ATPase receptor[24] was determined in a competitive binding assay,[25] employing [^{3}H]ouabain as displaced ligand. The IC_{50} values (concentration that inhibits ouabain binding by 50%) represent the means of values determined in two to three separate experiments in duplicate and were calculated using a nonlinear least-squares fitting algorithm.

(*E*)-(3β,14-Dihydroxy-5β,14β-androstane)-17β-acrylic Acid, 2-(*N*,*N*-Dimethylamino)ethyl Ester (5). To a suspension of NaH (0.078 g, 55% in oil, 1.79 mmol) in THF (2 mL) was added dropwise a solution of phosphonate **13** (554 mg, 2.07 mmol) in THF (3 mL) in 10 min. After 20 min of stirring, a solution of aldehyde **11**[17] monohydrate (500 mg, 1.48 mmol) in THF (5 mL) was added dropwise. The mixture was stirred for additional 30 min, and then the reaction was quenched by addition of ice/5% NaH_2PO_4 (50 mL) and extracted with EtOAc (3 × 50 mL) and $CHCl_3$ (2 × 50 mL). The combined organic phases were washed with brine, dried, and evaporated. The crude product was purified by FCC using $CHCl_3$/MeOH (9:1) as eluant to yield the unsaturated ester **5** as a white solid (540 mg, 84%). The product was then isolated as oxalate from EtOAc. Mp: 165−168 °C. IR (KBr): 1710, 1640, 1610 cm^{-1}. MS: m/z 433 (M^+). ^{1}H NMR (CD_3OD): δ 7.27 (1H, dd, J = 10.7, 15.5 Hz, H-20), 5.70 (1H, d, J = 15.5 Hz, H-21), 4.45 (2H, m, CH_2O), 4.07 (1H, m, H-3), 3.42 (2H, m, CH_2N), 2.90 (6H, s, NMe_2), 0.97 (3H, s, 10-CH_3), 0.84 (3H, s, 13-CH_3). ^{13}C NMR (CD_3OD): δ_C 16.6(q), 22.0(t), 22.4(t), 24.9(q), 27.9(t), 28.0(t), 28.6(t), 30.8(t), 33.1(t), 34.2(t), 36.5(s), 36.8(d), 37.5(d), 39.9(t), 42.7(d), 44.2(q), 50.6(s), 55.6(d), 57.7(t),

59.6(t), 67.7(d), 87.0(s), 119.1(d), 158.8(d), 167.2(s), 167.6(s). Anal. ($C_{26}H_{43}NO_4 \cdot C_2H_2O_4$) C, H, N.

(*E*)-(3*β*,14-Dihydroxy-5*β*,14*β*-androstane)-17α-acrylic Acid, 2-(*N*,*N*-Dimethylamino)ethyl Ester (6). Prepared in 61% yield from aldehyde **12** by the procedure described above for the preparation of **5**. Isolated as oxalate from EtOAc. Mp: 168−171 °C. IR (KBr): 1715, 1640, 1620 cm^{-1}. MS: *m*/*z* 433 (M^+). ^1H NMR (CD_3OD): δ 7.27 (1H, dd, J = 8.7, 15.5, H-20), 5.90 (1H, d, J = 15.5, H-21), 4.47 (2H, m, CH_2O), 4.05 (1H, m, H-3), 3.31 (2H, m, CH_2N), 2.85 (6H, s, NMe_2), 0.97 (3H, s, 10-CH_3), 0.94 (3H, s, 13-CH_3). ^{13}C NMR (CD_3OD): δ_C 17.9(q), 21.3(t), 22.1(t), 24.3(q), 26.0(t), 27.8(t), 28.6(t), 30.8(t), 31.8(t), 32.4(t), 34.2(t), 36.4(s), 36.9(d), 37.5(d), 42.5(d), 44.1(q), 50.6(s), 52.9(d), 57.7(t), 59.6(t), 67.7(d), 86.9(s), 121.6(d), 154.0(d), 167.2(s), 166.9(s). Anal. ($C_{26}H_{43}NO_4 \cdot C_2H_2O_4$) C, H, N.

(*E*)-(3*β*-Hydroxy-14-oxo-14,15-seco-5*β*-androstane)-17α-acrylic Acid, 2-(*N*,*N*-Dimethylamino)ethyl Ester (9). The crude 3-acetate (70 mg, 0.147 mmol), obtained from aldehyde **19** (78% yield) by the same procedure described above for the preparation of **5**, was hydrolyzed in 5% aqueous HCl/dioxane 1:2.5 (21 mL) at room temperature for 6 days. After removal of dioxane, the aqueous solution was extracted with EtOAc and the solvent evaporated. The crude product was purified by FCC using $CHCl_3$/MeOH (6:4) as eluant to yield the unsaturated ester **9** (58%). Viscous oil. IR (film): 1718, 1695 cm^{-1}. MS: *m*/*z* 433 (M^+). ^1H NMR ($CDCl_3$): δ 6.79 (dd, J = 10.5, 15.3 Hz, 1H, H-20), 5.89 (d, J = 15.7 Hz, 1H, H-21), 4.30 (m, 2H, CH_2O), 4.12 (m, 1H, H-3), 2.72 (m, 2H, CH_2N), 2.48 (m, 2H, H-8 and H-17), 2.38 (s, 6H, NMe_2), 1.08 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 0.85 (t, J = 6.5 Hz, 3H, CH_3CH$_2$). ^{13}C NMR ($CDCl_3$): δ_C 12.8(q), 20.3(t), 20.4(t), 23.1(t), 23.3(q), 24.3(q), 25.6(t), 27.9(t), 29.5(t), 32.6(t), 33.2(t), 35.9(s), 35.7(d), 40.1(d), 45.8(d), 45.4(q), 50.6(s), 51.1(d), 57.6(t), 61.5(t), 66.7(d), 123.4(d), 150.1(d), 166.3(s), 215.4(s). Anal. ($C_{26}H_{43}NO_4$) C, H, N.

(*E*)-(3*β*-Hydroxy-14-oxo-14,15-seco-5*β*-androstane)-17*β*-acrylic Acid, 2-(*N*,*N*-Dimethylamino)ethyl Ester (10). The compound was prepared by the same procedure described above for **9**, starting from aldehyde **20**. The crude product was purified by FCC using $CHCl_3$/MeOH (6:4) as eluant to yield the unsaturated ester as an inseparable *E*/*Z* (4:1) mixture (58% overall yield). Viscous oil. IR (film): 1715, 1695 cm^{-1}. MS: *m*/*z* 433 (M^+). ^1H NMR (major isomer, $CDCl_3$): δ 6.79 (dd, J = 10.5, 15.7 Hz, 1H, H-20), 5.83 (d, J = 15.7 Hz, 1H, H-21), 4.23 (m, 2H, CH_2O), 4.10 (m, 1H, H-3), 2.51 (m, 2H, CH_2N), 2.30 (s, 6H, NMe_2), 1.18 (s, 3H, 10-CH_3), 1.02 (s, 3H, 13-CH_3). ^{13}C NMR (major isomer, $CDCl_3$): δ_C 12.6(q), 18.8(q), 20.2(t), 20.4(t), 22.3(t), 23.3(q), 25.4(t), 27.8(t), 29.6(t), 33.1(t), 36.0(s), 35.7(d), 38.0(t), 41.9(d), 45.4(d), 45.6(q), 50.2(s), 51.1(d), 57.7(t), 61.9(t), 66.6(d), 123.2(d), 150.0(d), 166.4(s), 216.2(s). Anal. ($C_{26}H_{43}NO_4$) C, H, N.

3*β*,14-Dihydroxy-5*β*,14*β*-androstane-17α-carbaldehyde (12). To a solution of **11**[17] monohydrate (3.0 g, 8.88 mmol) in MeOH (45 mL) was added 1 N NaOH (42 mL). The solution was stirred overnight at room temperature. The white precipitate obtained by slow concentration of the solution was filtered and washed with MeOH/water (1:1) to give 2.55 g of **12** (85%). The product was crystallized from CH_3CN/water (7:3). Mp: 171−175 °C. ^1H NMR ($CDCl_3$): δ 9.82 (1H, d, J = 2.5, CHO), 4.13 (1H, m, H-3), 3.01 (1H, m, H-17), 1.25 (3H, s, 13-Me), 0.96 (3H, s, 10-Me).[14b]

Diethyl [2-(*N*,*N*-dimethylamino)ethoxycarbonyl]-methylphosphonate (13). To a solution of diethoxyphosphorylacetic acid (15 g, 76.5 mmol) and 2-(*N*,*N*-dimethylamino)-ethanol (7.8 mL, 76.5 mmol) in dry THF (250 mL) was added *N*,*N*′-dicyclohexylcarbodiimide (16.6 g, 80.3 mmol). After the mixture was stirred at room temperature for 6 days, the urea precipitate was filtered off, washing with THF, and the filtrate was evaporated to afford 20.1 g (98%) of the desired phosphonate **13** as a viscous yellow oil, which was sufficiently pure by TLC and ^1H NMR for use in the following reactions. MS: *m*/*z* 268 ($M + 1)^+$. ^1H NMR ($CDCl_3$): δ4.30 (2H, t, J = 7.0, OCH_2-CH$_2$), 4.18 (4H, quintet, J = 7.0, POCH_2CH$_3$), 3.02 (2H, d, J = 21.5, PCH_2CO), 2.70 (2H, m, CH_2N), 2.36 (6H, s, NMe_2), 1.35 (6H, t, J = 7.0, CH_2CH$_3$).

3*β*-Acetoxy-5*β*-pregn-14-ene (15). To a solution of 3*β*-acetoxy-14*β*-hydroxy-5*β*-pregn-20-one **14**[18] (12.9 g, 34.3 mmol) in pyridine (60 mL) at 0 °C was added in 20 min $SOCl_2$ (4.65 mL, 64.0 mmol). After 1 h the mixture was poured in iced water and extracted with EtOAc (3 × 150 mL). The organic layer was dried and evaporated. The crude product was purified by FCC using CH_2Cl_2/*n*-hexane (85:15) as eluant to give 3*β*-acetoxy-5*β*-pregn-14-en-20-one[28] (7.9 g, 64.2%) as a white solid. MS: *m*/*z* 358 (M^+). ^1H NMR ($CDCl_3$): δ 5.17 (1H, m, $W_{h/2}$ = 5.4, H-15), 5.08 (1H, m, $W_{h/2}$ = 7.5, H-3), 2.95 (1H, dd, J = 9.1, 7.5, H-17), 2.79 (1H, m, H-16), 2.18 (3H, s, CH_3-COC), 2.07 (3H, s, CH_3COO), 0.99 (3H, s, 10-CH_3), 0.87 (3H, s, 13-CH_3).

A solution of 3*β*-acetoxy-5*β*-pregn-14-en-20-one (7.9 g, 22.04 mmol) and *p*-toluenesulfonhydrazide (5.3 g, 28.65 mmol) in AcOH (80 mL) was stirred at room temperature for 4.5 h. After evaporation of the solvent, the crude product was dissolved in MeOH (150 mL) and zinc iodide[29] (1.09 g, 3.42 mmol) was added. To this solution was added portionwise sodium cyanoborohydride (2.7 g, 42.8 mmol), and the temperature was raised to the boiling point of the reaction mixture. After 4 h the reaction mixture was poured in 0.1 N NaOH/ice and extracted with EtOAc (4 × 150 mL); the organic layer was washed with water until neutral pH, dried, and evaporated. The crude product was purified by FCC using EtOAc/cyclohexane (96:4) as eluant to give **15** (5.9 g, 77.8%) as a white solid. MS: *m*/*z* 344 (M^+). ^1H NMR ($CDCl_3$): δ 5.19 (1H, m, $W_{h/2}$ = 5.4, H-15), 5.07 (1H, m, $W_{h/2}$ = 7.5, H-3), 2.38 (1H, m, H-16), 2.07 (3H, s, CH_3COO), 1.00 (3H, s, 10-CH_3), 0.93 (3H, t, J = 6.5 Hz, CH_3CH$_2$), 0.84 (3H, s, 13-CH_3). ^{13}C NMR ($CDCl_3$): 170.6(s), 155.6(s), 117.2(d), 70.7(d), 55.1(d), 46.9(s), 41.4(t), 40.2(d), 37.3(d), 36.3(t), 35.2(s), 35.1(d), 30.6(t), 30.5(t), 26.2(t), 25.1(t), 24.0(t), 23.6(q), 22.8(t), 21.8(t), 21.5(q), 17.5 (q), 13.4(q). Anal. ($C_{23}H_{36}O_2$) C, H.

3*β*-Acetoxy-5*β*,17α-pregn-14-ene (16). 3*β*-Acetoxy-14-hydroxy-5*β*,14*β*,17α-pregn-20-one[19] (9.1 g, 24.2 mmol) was first reduced to 3*β*-acetoxy-14-hydroxy-5*β*,14*β*,17α-pregnane via *p*-toluenesulfonylhydrazone and $NaBH_3CN/ZnI_2$, as described above for the preparation of **15**. The crude product was purified by FCC using *n*-hexane/EtOAc (82:18) as eluant to give 3*β*-acetoxy-14*β*-hydroxy-5*β*,17α-pregnane as a white solid (6.5 g, 73.8%). MS: *m*/*z* 362 (M^+). ^1H NMR ($CDCl_3$): δ 5.07 (1H, m, $W_{h/2}$ = 7.5, H-3), 2.05 (3H, s, CH_3COO), 0.98 (3H, s, 10-CH_3), 0.92 (3H, s, 13-CH_3), 0.89 (3H, t, J = 7.0, CH_3CH$_2$). ^{13}C NMR ($CDCl_3$): 170.6(s), 86.4(s), 70.6(d), 50.0(d), 46.4(s), 41.5(d), 37.0(d), 35.9(d), 35.1(s), 31.6(t), 30.6(t), 30.5(t), 30.0(t), 26.4(t), 25.9(t), 25.1(t), 23.8(q), 21.5(q), 22.3(t), 20.8(t), 20.3(t), 17.4(q), 13.4(q). This product was then dehydrated with $SOCl_2$ in pyridine as previously described to give **16** (5.1 g, 82.2%) as a white solid. MS: *m*/*z* 344 (M^+). ^1H NMR ($CDCl_3$): δ 5.10 (1H, m, $W_{h/2}$ = 5.4, H-15), 5.07 (1H, m, $W_{h/2}$ = 7.5, H-3), 2.51 (ddt, J = 15.0, 8.2, 2.0, H-16), 2.07 (3H, s, CH_3COO), 1.06 (3H, s, 13-CH_3), 1.02 (3H, s, 10-CH_3), 0.92 (3H, t, J = 7.0, CH_3-CH$_2$). ^{13}C NMR ($CDCl_3$): 170.6(s), 155.1(s), 115.7(d), 70.7(d), 51.3(d), 48.3(s), 42.7(d), 37.5(d), 36.6(t), 35.5(s), 35.2(d), 33.7(t), 30.6(t), 30.5(t), 26.4(t), 25.1(t), 25.0(q), 24.1(t), 23.7(q), 23.1(t), 21.3(t), 21.5(q),13.4(q). Anal. ($C_{23}H_{36}O_2$) C, H.

3*β*-Acetoxy-14-oxo-14,15-seco-5*β*-androstane-17α-carbaldehyde (19). A solution of **15** (5.4 g, 15.7 mmol) in CH_2Cl_2 (250 mL) was cooled at −78 °C, and a stream of ozone was passed through until the reaction was complete (ca.1 h). The excess of ozone was removed by a stream of nitrogen, then zinc (22.0 g) and AcOH (33.0 mL) were added portionwise, and the temperature of the reaction mixture was allowed to rise to room temperature. After 2 h of stirring the mixture was filtered and the solid was washed with CH_2Cl_2. The solution was then washed with NaOH (0.1 N) and water until neutral pH; the organic layer was dried and evaporated to give 3*β*-acetoxy-14,15-seco-14,15-dioxo-5*β*-pregnane **17** (5.8 g, 98%) as a solid sufficiently pure by TLC and ^1H and ^{13}C NMR for use in the subsequent reaction. MS: *m*/*z* 376 (M^+). ^1H NMR

($CDCl_3$): δ 9.82 (1H, t, $J = 2.4$, CHO), 5.08 (1H, m, $W_{h/2} = 7.5$, H-3), 2.57 (1H, dt, $J = 4.3, 11.5$, H-8), 2.49 (1H, ddd, $J = 1.6, 2.4, 16.5$, H-16), 2.33 (1H, m, H-17), 2.19 (1H, ddd, $J = 2.4, 5.9, 16.5$, H-16), 2.06 (3H, s, CH_3COO), 1.12 (3H, s, 13-CH_3), 1.04 (3H, s, 10-CH_3), 0.91 (3H, t, $J = 6.5$, CH_3CH$_2$). ^{13}C NMR ($CDCl_3$): δ_C 216.7(s), 203.2(d), 170.5(s), 70.2(d), 51.2(s), 46.1(t), 45.4(d), 41.0(d), 40.0(d), 36.5(d), 35.7(s), 35.1(t), 30.4(t), 30.2(t), 25.4(t), 25.0(t), 24.4(t), 23.2(q), 21.4(q), 20.5(t), 20.4(q), 20.2(t), 13.0(q). A solution of **17** (5.2 g, 13.83 mmol), Ac_2O (16.1 mL, 169.3 mmol), TEA (16.0 mL, 114.8 mmol), and DMAP (1.03 g, 8.43 mmol) in CH_2Cl_2 (43 mL) was stirred at room temperature for 48 h. The excess of Ac_2O was decomposed with EtOH (15 mL), and the solution was poured in iced water. The mixture was extracted with diethyl ether (3 × 100 mL), and the organic layer was washed with a 5% $NaHCO_3$ aqueous solution, dried, and evaporated. The crude 15-enolacetate mixture thus obtained was ozonized as previously described to give 0.51 g of crude **19** as a thick oil. This compound is scarcely stable and was used without any further purification in the subsequent Horner−Emmons reaction. ^{1}H NMR ($CDCl_3$): δ 9.90 (1H, d, $J = 3.0$, CHO), 5.08 (1H, m, $W_{h/2} = 7.5$, H-3), 2.58 (1H, dt, $J = 4.3, 11.5$, H-8), 2.38 (1H, m, H-17), 2.06 (3H, s, CH_3-COO), 1.30 (3H, s, 13-CH_3), 1.05 (3H, s, 10-CH_3), 0.95 (3H, t, $J = 6.5$, CH_3CH$_2$).

3β-Acetoxy-14-oxo-14,15-seco-5β-androstane-17β-carbaldehyde (20). Prepared in 24% overall yield from **16** using the same experimental procedures described previously for the preparation of **19**. This compound, obtained as a thick oil, is scarcely stable and was used without any further purification in the subsequent Horner−Emmons reaction. ^{1}H NMR ($CDCl_3$): δ 9.88 (1H, s, CHO), 5.09 (1H, m, $W_{h/2} = 7.5$, H-3), 2.52 (1H, dt, $J = 4.3, 11.5$, H-8), 2.07 (3H, s, CH_3COO), 1.23 (3H, s, 13-CH_3), 1.02 (3H, s, 10-CH_3), 0.93 (3H, t, $J = 7.0$, CH_3-CH$_2$).

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JM980108D