Cardenolide Analogues. 9. Synthesis and Biological Activity of 17β-Carbomethoxyethylene and 17β-Cyanoethylene 14α-H Steroids

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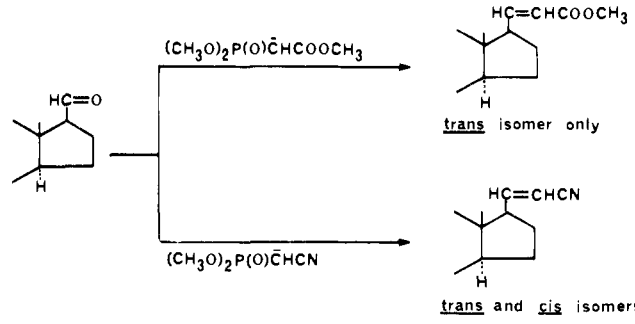
2-Carbomethoxyethylene and 2-cyanoethylene side chains were attached to the 17β position of a series of C/D trans steroids. The compounds were tested for digitalis-like activity and found to have no significant effects in doses ranging up to 10^{-4} M when tested for their ability to inhibit guinea pig (Na^+,K^+)ATPase and to affect guinea pig atrial contractility. In a previous study, it was shown that replacement of the lactone of digitoxigenin with the above side chains gave compounds with potent digitalis-like activity. The lack of activity in the present series implies that the carbomethoxyethylene and cyanoethylene side chains resemble the lactone in that they require the presence of a cis/trans/cis steroid in order to elicite biological activity. The compounds were prepared by reacting the appropriate phosphonate carbanion with the 17β-formyl derivatives of 3β-hydroxy-5β-androstane, 3β-hydroxyandrost-4-ene, 3α-hydroxy-5β-androstane, and 3β-hydroxy-5α-androstane. The reaction gave the trans isomer only of the carbomethoxyethylene derivatives and a mixture of the cis and trans isomers of the cyanoethylene derivatives.

The unique stereochemistry of the digitalis steroids seems to be essential for digitalis-like activity for compounds which have a 17β-lactone but is not essential for some other compounds with digitalis-like activity, such as the steroidal bisguanylhydrazones and the erythrophleum alkaloids.[1] It is thus possible to classify compounds with potent digitalis-like activity into those for which a 14β-OH steroid is essential and those for which it is not. In a previous study, we found that it was possible to replace the lactone of digitoxigenin with open-chain isosteres, such as the 17β-carbomethoxyethylene and 17β-cyanoethylene groups, to give compounds whose digitalis-like activity was similar to that of digitoxigenin.[2] In the present study, we have sought to determine whether these compounds fall into the class of digitalis-like compounds for which a 14β-OH steroid is essential. The hypothesis we wished to test was that rigid requirements regarding the stereochemistry of the steroid may not apply when the C-17 substituent was a flexible open-chain isostere of the lactone.

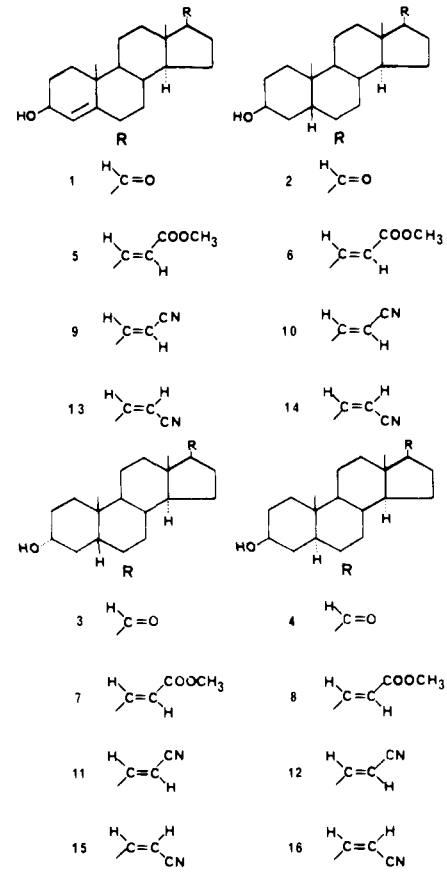
It should be explained that all previous work has shown that changing the 14β-OH of digitalis-like steroids to 14α-H produces an enormous reduction in biological activity, as illustrated by the recent work of Naidoo et al.[3] By contrast, the bisguanylhydrazones of steroids, such as prednisolone, show powerful digitalis-like activity in spite of the fact that they are 14α-H steroids. We have already pointed out[1] that the most likely reason for the high activity of the 14α-H bisguanylhydrazones was that the charged guanylhydrazone groups provided sufficient binding capacity to offset the unfavorable stereochemistry of the 14α-H steroids. Clearly, the 14β-OH is not a *general* prerequisite for digitalis-like activity, whereas an appropriate binding moiety at C-17 must be regarded, on present evidence, as an absolute requirement. The question considered in this report is whether the 14β-OH retains its relative importance when the lactone is replaced not with a charged moiety such as the guanylhydrazone group but with a side chain which resembles the lactone with respect to its electronic and stereochemical features but differs in that it is an open-chain moiety with increased capacity to make accommodating perturbations which may compensate for the altered stereochemistry of the steroid ring system.

Chemistry. The compounds were made by reacting the appropriate phosphonate carbanion with 17β-formyl steroids as shown in Scheme I. The preparation and proof of structure of the formyl steroids (1–4, Scheme II) were described elsewhere.[4]

Scheme I



Scheme II



The phosphonate carbanion, $(CH_3O)_2P(O)\bar{C}HCOOCH_3$, reacted smoothly with aldehydes 1–4 to give the (*E*)-α,β-unsaturated carbomethoxy esters 5–8 in good yield. The

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Table I. Nuclear Magnetic Resonance Data for Compounds 5–16[a]

no.	18-CH_3 (δ, mult)	19-CH_3 (δ, mult)	-OCH_3 (δ, mult)	3-CH (δ, mult, $W_{h/2}$)	21-CH (δ, mult, $J_{20,21}$)	20-CH (δ, mult, $J_{17,20}$, $J_{20,21}$)	4-CH (δ, mult, $W_{h/2}$)
5	0.66, s	1.04, s	3.71, s	4.13, m, 11.0	5.79, d, 15.35	7.00, dd, 7.3, 15.3	5.31, m, 4.0
6	0.64, s	0.99, s	3.76, s	4.16, m, 5.3	5.83, d, 15.3	7.07, dd, 7.3, 15.3	
7	0.64, s	0.95, s	3.77, s	*b*	5.82, d, 15.35	7.03, dd, 7.3, 15.35	
8	0.65, s	0.83, s	3.75, s	*b*	5.82, d, 15.4	7.03, dd, 7.3, 15.4	
9	0.67, s	1.06, s		4.17, m, 14.5	5.27, d, 16.7	6.74, dd, 7.3, 16.7	5.31, m, 3.3
13	0.72, s	1.06, s		4.17, m, 14.5	5.39, d, 11.3	6.51, t, 11.3, 11.3	5.31, m, 3.3
10	0.66, s	0.99, s		4.15, m, 7.3	5.30, d, 16.0	6.74, dd, 8.0, 16.0	
14[c]	0.70, s	0.99, s		4.15, m, 7.3	5.36, d, 11.3	6.48, t, 11.3, 11.3	
11	0.62, s	0.93, s		3.62, m, 20	5.33, d, 16.0	6.74, dd, 7.3, 16.0	
15	0.67, s	0.93, s		3.62, m, 20	5.35, d, 11.3	6.47, t, 11.3, 11.3	
12	0.64, s	0.82, s		3.59, m, 20	5.32, d, 16.0	6.82, dd, 8.0, 16.0	
16[c]	0.68, s	0.82, s		3.59, m, 20	5.38, d, 11.3	6.49, t, 11.3, 11.3	

[a] NMR spectra were determined in $CDCl_3$. Abbreviations used: mult, multiplicity; s, singlet; d, doublet; t, triplet; dd, doublet of doublet; m, multiplet. [b] 3-CH signal is concealed by the -OCH_3 signal. [c] The spectrum was determined on a mixture consisting of approximately equal amounts of both isomers.

trans configuration of 5–8 was confirmed by NMR spectroscopy. A well-defined doublet, centered at δ 5.79–5.83, was assigned to the proton at C-21. The high coupling constant ($J_{20,21}$ = 15.3–15.4 Hz) was indicative of a trans relationship between the protons on C-20 and C-21. The origin of the signal at δ 5.79–5.83 was confirmed by comparison of the NMR spectrum of (*E*)-21-carbomethoxy-3β,14β-dihydroxy-5β-pregn-20-ene, which displayed a doublet centered at δ 5.61 ($J_{20,21}$ = 15.5 Hz), with the homologous 21-methyl compound, which did not give a signal in the region of δ 5.61.[5] Because of resonance effects, the C-20 olefinic proton has a partial positive charge and is more deshielded than the C-21 proton. Its NMR signal therefore appeard as a pair of doublets centered at δ 7.00–7.07. The doublets arose from coupling with the proton at C-21 and further splitting by the proton at C-17 ($J_{17,20}$ = 7.3 Hz). There were no extraneous lines in the olefinic region which could be attributed to the presence of the cis isomer.

Condensation of $(CH_3O)_2P(O)\bar{C}HCN$ with aldehydes 1–4 gave mixtures of the α,β-unsaturated *cis*- and *trans*-nitriles 9–16. In every case, isolation of the pure trans isomer was achieved by fractional crystallization. In some cases, the cis isomer was also isolated by careful recrystallization from methanol. The trans isomer was invariably less soluble in methanol and had a higher melting point than the cis isomer. The unsaturated *cis*- and *trans*-nitriles were identified by NMR spectroscopy. The olefinic protons of the trans isomers showed splitting patterns very similar to those of the *trans*-methyl esters 5–8, whereas the double-bond protons of the cis isomers gave a five-line splitting pattern comprising a doublet and a triplet. The doublet, centered at δ 5.35–5.39, was assigned to the proton at C-21, cis coupled with the proton at C-20 ($J_{20,21}$ = 11.3 Hz). As expected from Karplus' considerations,[6] J_{trans} was greater than J_{cis} (15.3 as compared with 11.3 Hz). The triplet at δ 6.47–6.51 was assigned to the proton on C-20 coupled with the proton on C-21 and further split by the proton on C-17 ($J_{17,20}$ = 11.3 Hz).

The observed differences in the chemical shifts of the C-20 proton of the cis and trans olefins (Table I) is in keeping with published NMR data for α,β-unsaturated nitriles[7] and adds further support for the present assignment of cis and trans configurations. The different magnitudes of $J_{17,21}$ for the *cis*- and *trans*-nitrile compounds suggest that the orientation of substituents about the C-20,21 double bond leads to steric interactions which alter the dihedral angle between protons on C-17 and C-20.

For the compounds synthesized, the chemical shift of the methyl signal furthest upfield was affected by the C-17 substituent to a greater extent than the downfield methyl signal. This supports the NMR assignment of the 18- and the 19-CH_3 signals and is consistent with the α configuration of the proton at C-14.

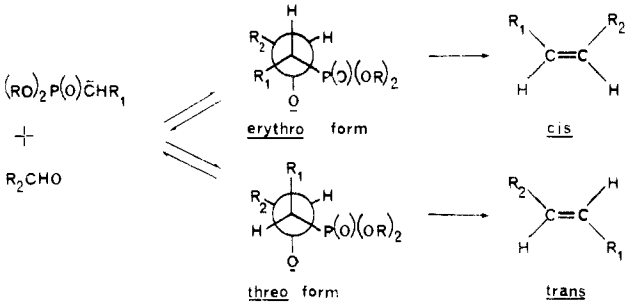

Figure 1. Mechanism for olefin formation by stabilized phosphonate carbanions.

The chemistry employed in the synthesis of the 17β-formyl steroids[4] and their subsequent condensation to give the title compounds is unlikely to cause epimerization at C-17. The assignment of β-orientation to the side chain at C-17 is substantiated by the position of the 19- and the 18-CH_3 signals in the NMR spectra. For 17α-epimers, the signals would be expected to be almost coincidental, giving rise to a single diffuse band, or for 18-CH_3 to appear downfield from the 19-CH_3 signal.[8]

The mechanism for olefin formation by stabilized phosphonate carbanions has been reviewed by Boutagy and Thomas.[9] Briefly, the phosphonate carbanion is formed under the influence of strong base. The addition of the carbanion to the carbonyl carbon proceeds through the formation of a reversible oxyanion which decomposes to the olefin through cis elimination of the phosphate ion. The stereochemical course of the reaction is influenced by the stereochemistry of the oxyanion, which can exist in two diastereoisomeric forms (Figure 1). Preference for the threo or the erythro form is dependent on the steric crowding of the substituent groups. Approximately equal quantities of both cis and trans olefins were obtained when R_1 = CN. The linear, sp-bonded nitrile group is small in bulk and spatially placed so that both the threo and erythro forms should be equally possible. Only the trans olefin was formed when R_1 = $COOCH_3$. The carbomethoxy group is considerably more bulky than the nitrile group and presumably the formation of the threo oxyanion was favored.

Biological Activity. In a previous study we established that the lactone of digitoxigenin could be replaced with carbomethoxyethylene and cyanoethylene groups to give potent digitalis-like compounds.[2] In the present study we found that attachment of the above side chains to the 17β position of various 14α-H steroids gave a series of compounds (5–16) which had no significant effects on guinea

pig atrial contractility or on guinea pig myocardial (Na^+,K^+)ATPase when used in concentrations ranging up to 10^{-4} M. Thus, in spite of the increased flexibility due to the open-chain nature of the lactone isosteres and the altered binding possibilities associated with the cis isomers, it would seem that the forces capable of binding the carbomethoxyethylene and cyanoethylene groups to the receptor are such that reinforcement is required in the form of a stereospecific set of interactions involving the steroid ring system.

Experimental Section

Chemistry. General Methods. Melting points were determined on a Kofler hot block and are uncorrected. IR spectra were recorded on a Perkin-Elmer Model 21 double-beam spectrophotometer or on a Unicam SP200/G spectrophotometer. NMR spectra were recorded by a Varian A-60 instrument using Me_4Si (δ 0.00) as internal standard. The values of δ (ppm) are quoted for the 60-MHz instrument. Mass spectra were obtained on an AEI MS9 instrument operating at a 12- or 70-eV ion current with the source several degrees below the melting point of the compounds. Microanalyses were carried out by the Australian Microanalytical Service, Melbourne. Analytical samples were dried at 40–50 °C (0.5 mmHg) over P_2O_5 and paraffin for 24 h. Where water of crystallization was indicated, the samples were further dried at elevated temperatures for 24 h. Where analyses are indicated by symbols of the elements, the results were within ±0.4% of the theoretical values.

Synthesis of 17β-Carbomethoxyethylene Steroids 5–8. These compounds were prepared using the following ratios of material. Trimethyl phosphonoacetate (5 mmol) was added dropwise with stirring into a dry flask containing oil-free NaH (5 mmol) suspended in 1,2-dimethoxyethane (DME; 25 mL). The mixture was stirred at room temperature with exclusion of moisture for 20 min, by which time evolution of H_2 had ceased. The appropriate 17β-formyl steroid (1–4; 1 mmol) dissolved in DME (15 mL) was added, and the mixture was stirred at room temperature for 2 h, by which time all the carbonyl compound had reacted with the phosphonate (the reaction mixture gave a negative reaction with DNP reagent at 2 h). Water (10 mL) was added, and most of the DME was removed under reduced pressure at 25 °C. The solid then separated and was collected by filtration, washed with H_2O, and recrystallized from the appropriate solvent. The following compounds were synthesized by the above method.

(*E*)-21-(Methoxycarbonyl)-3β-hydroxypregna-4,20-diene (5) was obtained in 89% yield: recrystallized from $EtOEt/C_5H_{12}$; mp 102–104 °C; IR ν_{max} (Nujol) 3400 (OH), 1720 (CO), 1650 (C=C), 1255, 1245 (C-OAc) cm^{-1}; mass spectrum m/e 358 (M^+). Anal. ($C_{23}H_{34}O_3 \cdot 0.5H_2O$) C, H.

(*E*)-21-(Methoxycarbonyl)-3β-hydroxy-5β-pregn-20-ene (6) was obtained in 78% yield: recrystallized from C_6H_{12}; mp 106–109 °C; IR ν_{max} (Nujol) 3620 (OH), 1730 (CO), 1650 (C=C), 1260, 1245 (C-OAc) cm^{-1}; mass spectrum m/e 360 (M^+). Anal. ($C_{23}H_{36}O_3$) C, H.

(*E*)-21-(Methoxycarbonyl)-3α-hydroxy-5β-pregn-20-ene (7) was obtained in 75% yield: recrystallized from C_6H_{12}/Me_2CO; mp 121–125 °C; IR ν_{max} (Nujol) 3360 (OH), 1730 (CO), 1660 (C=C), 1260, 1245 (C-OAc) cm^{-1}; mass spectrum m/e 360 (M^+). Anal. ($C_{23}H_{36}O_3$) C, H.

(*E*)-21-(Methoxycarbonyl)-3β-hydroxy-5α-pregn-20-ene (8) was obtained in 70% yield: recrystallized from C_6H_{12}; mp 87 °C; IR ν_{max} (Nujol) 3360 (OH), 1720 (CO), 1660 (C=C), 1260, 1250 (C-OAc) cm^{-1}; mass spectrum m/e 360 (M^+). Anal. ($C_{23}H_{36}O_3$) C, H. NMR spectral data for compounds 5–8 are given in Table I.

Synthesis of 17β-Cyanoethylene Steroids 9–16. These compounds were prepared using the following ratios of material. Diethyl cyanomethylphosphonate (6.7 mmol) was added dropwise with stirring into a dry flask containing oil-free NaH (6.7 mmol) suspended in DME (15 mL). The mixture was stirred at room temperature, with exclusion of moisture, for 20 min, by which time evolution of H_2 had ceased. The appropriate 17β-formyl steroid 1–4 (1.3 mmol) dissolved in DME (20 mL) was added, and the mixture was stirred at room temperature for 30 min, by which time all the carbonyl compound had reacted (negative DNP test). Water (10 mL) was added, and most of the DME was removed under reduced pressure at 25 °C. The solid then separated and was collected by filtration, washed with H_2O, dried, and recrystallized from MeOH. The reaction with each 17β-formyl steroid gave a mixture of geometric isomers about the C-20,21 double bond. The trans (*E*) isomer was isolated in each instance by careful recrystallization from MeOH. Sometimes, the pure cis (*Z*) isomer was also obtained by the same method. The following compounds were synthesized by the above method.

(*E*)-21-Cyano-3β-hydroxypregna-4,20-diene (9): mp 220 °C; IR ν_{max} (Nujol) 3480 (OH), 2220 (CN), 1650, 1625 (C=C) cm^{-1}; mass spectrum m/e 325 (M^+). Anal. ($C_{22}H_{31}NO$) C, H, N.

(*Z*)-21-Cyano-3β-hydroxypregna-4,20-diene (13): mp 152–154 °C; IR ν_{max} (Nujol) 3490–3200 (OH), 2220 (CN), 1650, 1625 (C=C) cm^{-1}; mass spectrum m/e 325 (M^+). Anal. ($C_{22}H_{31}NO \cdot 0.5H_2O$) C, H, N. Compounds 9 and 13 were obtained in a combined yield of 86%.

(*E*)-21-Cyano-3β-hydroxy-5β-pregn-20-ene (10): mp 155–157 °C; IR ν_{max} (Nujol) 3300 (OH), 2220 (CN), 1655 (C=C) cm^{-1}; mass spectrum m/e 327 (M^+). Anal. ($C_{22}H_{33}NO$) C, H, N.

(*Z*)-21-Cyano-3β-hydroxy-5β-pregn-20-ene (14). This compound was not separated from 10. The mixture of approximately equal amounts of 10 and 14 melted at 125–130 °C. Elemental analyses of the mixture were the same as for 10. Compounds 10 and 14 were obtained in a combined yield of 79%.

(*E*)-21-Cyano-3α-hydroxy-5β-pregn-20-ene (11): mp 185 °C; IR ν_{max} (Nujol) 3520 (OH), 2230 (CN), 1640 (C=C) cm^{-1}; mass spectrum m/e 327 (M^+). Anal. ($C_{22}H_{33}NO$) C, H, N.

(*Z*)-21-Cyano-3α-hydroxy-5β-pregn-20-ene (15): mp 145 °C; IR ν_{max} (Nujol) 3500 (OH), 2220 (CN), 1660 (C=C) cm^{-1}; mass spectrum m/e 327 (M^+). Anal. ($C_{22}H_{33}NO$) C, H, N. Compounds 11 and 15 were obtained in a combined yield of 82%.

(*E*)-21-Cyano-3β-hydroxy-5α-pregn-20-ene (12): mp 187–190 °C; IR ν_{max} (Nujol) 3495 (OH), 2220 (CN), 1635 (C=C) cm^{-1}; mass spectrum m/e 327 (M^+). Anal. ($C_{22}H_{33}NO$) C, H, N.

(*Z*)-21-Cyano-3β-hydroxy-5α-pregn-20-ene (16). This compound was not separated from 12. The mixture of approximately equal amounts of 12 and 16 melted at 150 °C. Elemental analyses of the mixture were the same as for 12. Compounds 12 and 16 were obtained in a combined yield of 79%. NMR spectral data for compounds 9–16 are given in Table I.

Biological activity was determined as previously described.[2,3,9]

Inhibition of Phosphohydrolase Activity. (Na^+,K^+)ATPase and Mg^{2+}-ATPase were prepared from guinea pig myocardium as previously described.[9] The crude membrane preparations contained 5–10 units of each enzyme activity. Units of activity were calculated as micromoles of inorganic phosphate liberated per milligram of protein per hour. Incubations were carried out at 37 °C for 30 min as previously described.[9] Mg^{2+}-ATPase activity (with and without drug) was determined in the absence of Na^+ and K^+ and was ouabain insensitive. (Na^+,K^+)ATPase activity was determined by subtracting Mg^{2+}-ATPase activity from total phosphohydrolase activity and was completely inhibited by 10^{-4} M ouabain. (Na^+,K^+)ATPase activity in the presence of drug was obtained by subtraction of Mg^{2+}-ATPase activity in the presence of drug from total phosphohydrolase activity in the presence of the same concentration of drug. All measurements were corrected for nonenzymatic hydrolysis of ATP. The rate of release of inorganic phosphate in the absence of drug was pseudo-first-order when followed for 60 min. The total amount of substrate consumed in 30 min in the absence of drug was less than 15% of the total available. Time-dependence effects on rate in the presence of drug were not examined.

The dose-response relationship for the test compounds was determined over a drug concentration range of 10^{-8}–10^{-4} M. All determinations were carried out in duplicate.

Determination of Inotropic Activity. Inotropic activity was determined using the left auricle of the guinea pig as previously described.[2] The auricle was suspended in Krebs–Henseleit solution at 32 °C and was gassed with O_2 containing 5% CO_2. The resting tension was about 1 g. The auricle was stimulated by a rectangular pulse (10-ms duration) at a frequency of 100/min. The voltage was set at 20% above threshold. Changes in isometric contraction were calculated with reference to changes in control atria using the protocol described previously.[2] The dose–response relationship for the test compounds was determined over a drug

concentration range of 10^{-8}–10^{-4} M.

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Studies on Antianaphylactic Agents. 6.[1] Synthesis of Some Metabolites of 6-Ethyl-3-(1*H*-tetrazol-5-yl)chromone and Their Analogues

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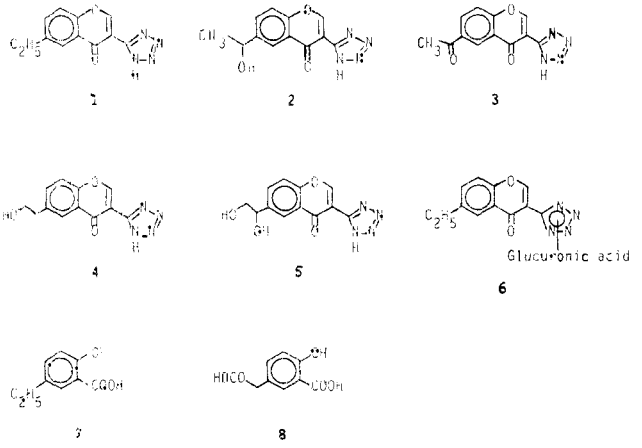
The metabolites of 6-ethyl-3-(1*H*-tetrazol-5-yl)chromone (AA-344) (1), an orally effective antiallergic agent, and their analogues were synthesized to confirm the proposed structures and to determine their activity in the rat passive cutaneous anaphylaxis (PCA) test. A glucuronic acid metabolite (6) was assigned the structure **24b**, 1-deoxy-1-[5-(6-ethylchromon-3-yl)tetrazol-1-yl]-β-D-glucopyranuronate, by the comparison of ^{13}C NMR, mass spectra, and TLC of isomeric compounds. In ^{13}C NMR spectra, the shift difference of the tetrazole ring carbons between a pair of isomers was more remarkable than that of the glycosidic carbons. Therefore, the former is a useful criterion for distinguishing between such isomers. Some of the metabolities and analogues were active when administered intravenously, and two metabolites (**2** and **3**) were also effective upon oral administration.

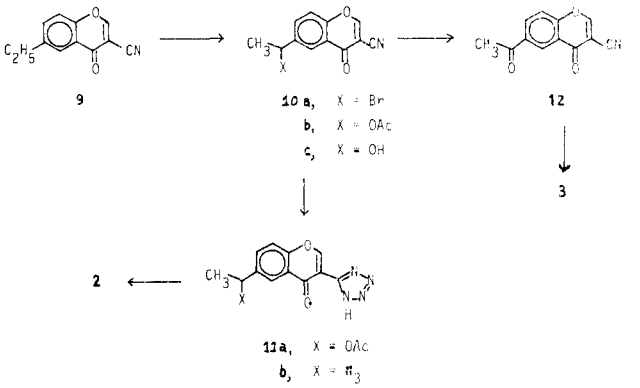
3-(1*H*-Tetrazol-5-yl)chromones have been shown to inhibit homologous passive cutaneous anaphylaxis (PCA) reactions induced by reaginic antibody in the rat, when administered intravenously or orally.[1] After examining the pharmacological and toxicological properties of a variety of drugs, 6-ethyl-3-(1*H*-tetrazol-5-yl)chromone (AA-344) (**1**) was selected as one of the most promising, and its



metabolic fate was investigated. In the metabolism study,[2] seven metabolites, 6-(1-hydroxyethyl)- (**2**), 6-acetyl- (**3**), 6-(2-hydroxyethyl)- (**4**), and 6-(1,2-dihydroxyethyl)-3-(1*H*-tetrazol-5-yl)chromone (**5**), glucuronide (**6**), 5-ethylsalicylic acid (**7**), and 3-carboxy-4-hydroxyphenylacetic acid (**8**) were tentatively identified in the urine of rats, guinea pigs, rabbits, dogs, and monkeys. These seven metabolites and analogues of **2** and **3** were synthesized in an effort to unequivocally assign structures to them and to allow evaluation of their antiallergic activity.

Chemistry. The metabolites 6-(1-hydroxyethyl)- (**2**) and 6-acetyl-3-(1*H*-tetrazol-5-yl)chromone (**3**) were synthesized by the two routes shown in Schemes I and II. Initially, the routes from 6-ethyl-4-oxo-4*H*-1-benzopyran-3-carbonitrile (**9**)[1] were attempted. Bromination of **9**[1] with *N*-bromosuccinimide (NBS), followed by a substitution reaction with sodium acetate, gave the acetoxy derivative **10b**. Reaction of **10b** with sodium azide in the presence of anhydrous aluminum chloride in tetrahydrofuran[3] gave both the acetoxy tetrazole **11a** and the azide derivative **11b**. Hydrolysis of the acetoxy derivative **11a** with 1 N NaOH at room temperature gave the metabolite **2**. The hydrolysis of either **10a** or **10b** with aqueous alkali gave the hydroxy derivative **10c**, which was oxidized to the ketone **12** with Jones reagent.[4] Compound **12** was converted to the metabolite **3** by the tetrazole ring synthesis described above (Scheme I).

Scheme I



An alternative and more convenient route to **2** and **3** involves the bromination of **1** as shown in Scheme II. Bromination of **1** with NBS, followed by alkaline hydrolysis, gave **2**, which was converted to **3** by Jones oxidation. In a similar manner, analogues of metabolites

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