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ACYLATION OF THE 4,5- AND 5,6-DOUBLE BOND ISOMERS OF 3-STEROIDAL THIAZOLIDINES

THE CHEMICAL STABILITY OF SPIRO-3-STEROIDAL 4,5- AND 5,6-DOUBLE BOND ISOMERS

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Abstract—It has been shown that the 5,6-double bond steroidal thiazolidines can be N-acylated if there is no substituent in the 4'-position on the thiazolidine ring; substituents in the 4'-position of the thiazolidine sterically hinder acylation. On the other hand, the 4,5-double bond steroidal thiazolidine isomers decomposed on attempted acylation. The lability of these 4,5-double bond isomers was attributed to the contribution of a hyperconjugative resonance form to the structure of the 4,5-double bond isomers.

Thiazolidines are an important ring system in biology and medicine. Not only do they constitute the backbone of the penicillin structure, but they also almost certainly form *in vivo* from the reversible[1] reactions of cysteine with endogenous aldehydes such as pyridoxal[2] or the aldehydes involved in collagen crosslinking.[3] Reversibility in these biological reactions is usually advantageous. On the other hand, reversibility, if it is too facile, can be a liability especially if the thiazolidine is to be used as an intermediate in synthetic schemes or as a CO protecting group. Thus, it has frequently been necessary to accylate the thiazolidine after it has been formed so that it will be resistant to subsequent chemical reactions.[4,5]

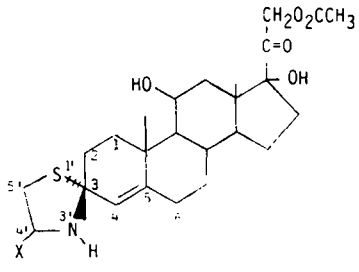
Recently, the synthesis and biological activity of the previously unknown thiazolidines of α,β-unsaturated steroidal ketones were reported.[6] These thiazolidines were found to be too labile for their intended use as prodrugs[6d] in oral dosage forms[6c] and N-acylation was investigated as a means of stabilizing them. The following is a report of that investigation and related observations on the relative stability of 4,5- and 5,6-double bond 3-spiro steroidal ketone derivatives.

Previously reported N-acylations of thiazolidines have been high yield reactions,[5] thus, the initial results from the reaction of thiazolidine **1** with acetic anhydride in CH_2Cl_2 or pyridine were unexpected. The NMR spectrum of the crude reaction mixture showed the complete loss of the characteristic thiazolidine steroidal C$\underline{H}$=C absorption at δ 5.23 after 1 hr, and after 24 hr tlc analysis showed that the mixture contained primarily hydrocortisone 21-acetate. The more vigorous reaction conditions (Ac_2O in H_2O at 100° or AcOH-Ac_2O in pyridine) reported for the N-acylation 2-arylthiazolidines[5] were not even attempted with **1** or the other 4,5-double bond isomers in view of this unexpectedly facile decomposition under ordinarily mild conditions. The remaining exploratory reactions were run in NMR spin tubes (approximately 50 mg of thiazolidine dissolved in 2 ml of $CDCl_3$ and 20 mg of acetic anhydride) and monitored by NMR spectroscopy and tlc.

The results from the exploratory reactions of the double bond isomers **2** and **3** suggested that the position of the double bond influenced the course of the reaction. The reaction with the 4,5-double bond isomer **2** proceeded very quickly, but the thiazolidine ring was destroyed in the process. The C$\underline{H}$=C absorption attributable to the thiazolidine was almost completely gone after 1 hr; the C$\underline{H}$=C absorption was shifted downfield from δ 5.3 to the region expected for an N=C–C$\underline{H}$=C type absorption and was split into two sharp singlet absorptions at δ 6.0 and 5.77. For comparison, the ethanolamine imine of testosterone[7] exhibited C$\underline{H}$=C absorptions at δ 5.97 and 5.77. On the other hand, the reaction of acetic anhydride with the 5,6-double bond isomer **3** was very slow. No discernable reaction at all was apparent after 1 hr and after 24 hr at least 60% of the thiazolidine steroidal C$\underline{H}$=C absorption was intact. The remaining material appeared to be testosterone since the C$\underline{H}_3$–C and C$\underline{H}$=C absorptions attributed to testosterone were enhanced after the sample was spiked with testosterone. Tlc confirmed that the two main components in the reaction mixture were the starting thiazolidine and testosterone. The reaction of the analogous series of progesterone thiazolidine double bond isomers exhibited similar behavior.

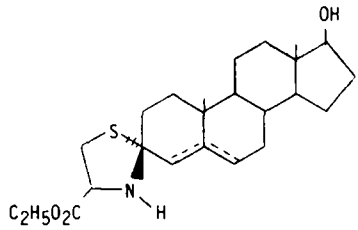
The reaction of hydrocortisone 21-acetate thiazolidine **4** (a 4,5-double bond isomer) resulted in complete and extremely rapid decomposition of **4** as soon as the acetic anhydride was added. The more vigorous reaction conditions referred to earlier (heat, pyridine) caused increased hydrolysis of the 5,6-double bond isomer **3** rather than N-acylation. Thus, 4,5-double bond steroidal thiazolidines regardless of substitution in 4'-position rapidly degraded when allowed to react with acetic anhydride while the 5,6-double bond isomer with a 4'-carboethoxy group reacted very slowly and then did not afford an acylated product.

Only in those cases where there was no 4'-substituent on the thiazolidine ring and the double bond was in the 5,6-position was any N-acylthiazolidine obtained. Thus, when the progesterone thiazolidine **5** was allowed to react with acetic anhydride in CH_2Cl_2 for 24 hr, a 51% yield of acetate **6** (m.p. 201–203°) was obtained. Sub-



1 X = $CO_2C_2H_5$

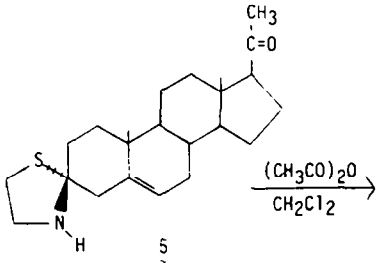
4 X = H



2 4,5-double bond

3 5,6-double bond

stitution of N-carbobenzyloxyglycine anhydride[8] or formic-acetic anhydride[9] for acetic anhydride in the reaction afforded **7** and **8**, respectively.



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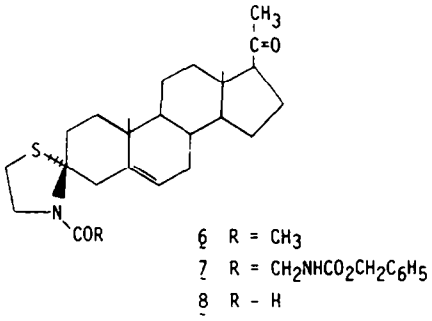
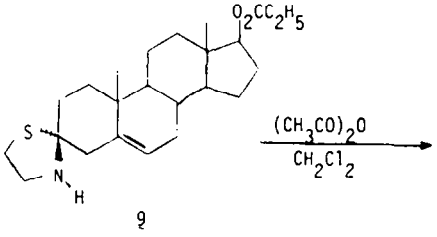


6 R = CH_3

7 R = $CH_2NHCO_2CH_2C_6H_5$

8 R - H

Similarly, testosterone 17β-propionate thiazolidine **9** gave N-acylated thiazolidine **10** when it was allowed to react with acetic anhydride.



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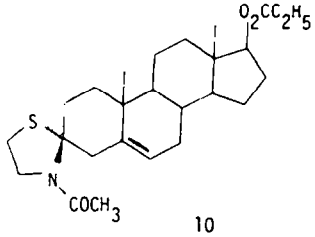


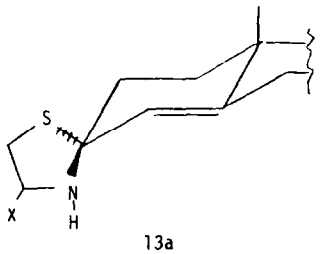
10

It was confirmed that no acylthiazolidines had been formed in the exploratory reactions, when the spectra from the exploratory reactions were re-examined in the light of the NMR spectra and tlc of the N-acylthiazolidines **6**, **7**, **8** and **10**. For instance, the spectra of **6**, **7** and **8** showed that the CH=C absorption in **5** did not shift upon N-acylation while the 19-CH_3 absorption consistently underwent a downfield shift of about 4 cps in going from **5** to **6**, **7** or **8**, and the CH–N (X = H) absorption experienced a downfield shift of about 35 cps. On the other hand, the thiazolidine steroidal CH=C absorption of **1**, **2** or **4** in the exploratory NMR experiments either disappeared completely or, if that absorption remained intact (in **3**), the 19-CH_3 and the CHX–N (X = CO_2Et) absorptions did not shift.

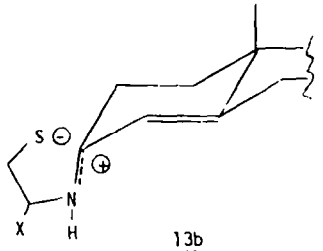
The positions of the double bonds in **6**, **7** and **8** were determined by ^{13}C NMR spectroscopy. Their spectra showed olefinic carbon absorptions at about δ 148 and δ 120, which were consistent with the previously determined trend for 5,6-double bond isomers.[10]

The fact that 2,2-dimethyl-1,3-thiazolidine-4-carboxylic acid (**11**),[4] cyclohexane spiro-1,2'-(1',3'-thiazolidine-4'-carboxylic acid) (**12**)[4] and the 5,6-double bond isomers **5** and **9** were acylated under conditions which led to decompostition of the 4,5-double bond thiazolidines **1**, **2** and **4** suggested a destabilizing effect of the 4,5-double bond on the thiazolidine ring. This destabilizing effect may be due to the known "loosening"[11] of bonds adjacent to double bonds exemplified by the well-known increased reactivity of allylic halides. Thus, the actual structure of **13a** may contain a significant contribution from the hyperconjugative structure **13b** where the nucleophilicity of the nitrogen effectively is decreased while the nucleophilicity of sulfur is increased. Moreover, since the thiazolidine ring is already subject to a facile ring opening-ring closing equilibrium during acylation,[5] it is not surprising that the introduction of α,β-double bond in the thiazolidine structure, which would further stabilize the ring opened form, causes a shift of the reaction away from N-acylation. Thus, sulfur

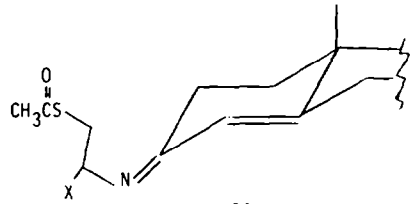
instead of nitrogen in the 4,5-double bond isomers should be acylated under the reaction conditions to give **14**. However, although ^{1}H NMR spectra of NMR spin tube reactions between the 4,5-double bond isomers and acetic anhydride indicated the transient formation of absorptions that could be attributed to C=CH–C=N[7] type moieties, **14** could not be isolated from larger scale preparative reactions.



13a



13b



14

The relative instability of the 4,5-double bond isomer compared to the 5,6-double bond isomer that was found in the thiazolidine series also was observed in the steroidal ethylene ketal and hemithioketal series. Peterson and Sowers[12] reported that the 5-cholesten-7-one and 4-cholestene-3-one ethylene ketals were extremely sensitive to hydrolysis and had to be stored over pyridine. We have observed a similar sensitivity displayed by the 4,5-double bond isomers of testosterone propionate thiazolidine,[6c] ethylene ketal[10,13] and hemithioketal.[10] Thus the 4,5-double bond isomers, except for the thioketal, were all observed to decompose slowly, even in the solid state, to give the parent α,β-unsaturated steroid, while the 5,6-double bond ketals and hemithoiketals were found unchanged after several years at room temperature.

Thus, the success of the acylation of the 4,5- and 5,6-double bond isomers of steroidal thiazolidines depended on the position of the double bond and the substitution on the thiazolidine ring. The 4,5-double bond isomers were too labile to be acylated under the conditions used and the 4'-carboalkoxy group on the thiazolidine and steroid together provided too much steric hindrance to N-acylation of the 5,6-double bond isomers. Consequently, only the 4'-unsubstituted thiazolidine of the 5,6-double bond steroid isomer could be acylated.

EXPERIMENTAL

Tlc were run on Brinkman Polygram Sil G/UV 254; ether. M.p. (uncorrected) were taken with a Thomas-Hoover capillary apparatus. NMR spectra were recorded on a Varian T-60 (^{1}H spectra) or on a Bruker WP-80 (^{13}C spectra) at the University of Kansas. IR spectra were obtained on a Beckman Accu-Lab 4 infrared spectrophotometer. Microanalyses were performed by Midwest Microlab, Ltd., Indianapolis, Indiana. The hydrocortisone 21-acetate, testosterone, progesterone and cysteine ethyl ester hydrochloride were obtained from Sigma. 2-Aminoethanethiol hydrochloride was obtained from Aldrich. The thiazolidine reactants in the acylation reactions were prepared according to methods described previously,[6] from the reaction of the steroids with the amino alkythiol hydrochlorides in pyridine.

Preparation of 5-pregenene-20-one-3-spiro-2'-(3'-acetyl-1',3'-thiazolidine) (**6**). 5-Pregnene-20-one-3-spiro-2'-(1',3'-thiazolidine) (0.8 g, 0.00214 mol) was dissolved in 10 ml CH_2Cl_2 and allowed to react with 0.6 g (0.006 mol) Ac_2O for 24 hr. The soln was diluted with CH_2Cl_2 (75 ml), washed with water (50 ml), dried over Na_2SO_4 and concentrated *in vacuo* to give a yellow gum. The gum was crystallized from 3 ml of MeOH to give 0.30 g (m.p. 201–203°, 34% yield) of the desired compound: tlc (silica gel, ether) R_f 0.15; IR (KBr) 1700 cm^{-1} (s) (C=O) and 1650 cm^{-1} (s) (C=O); ^{1}H NMR ($CDCl_3$) δ 5.47–5.2 (m, 1, C$\underline{H}$=C), 3.9 (t, 2, J=6H$_z$, C$\underline{H}_2$–N), 2.83 (t, 2, J=6H$_z$, C$\underline{H}_2$–S), 2.1 (s, 6, CH_3CON and $C\underline{H}_3CO_2$), 1.1 (s, 3, C$\underline{H}_3$–C), 0.63 (s, 3, C$\underline{H}_3$–C), and 3.5–1.0 (m, 20, C$\underline{H}_2$ and C$\underline{H}$); ^{13}C NMR ($CDCl_3$) δ 209.4 (C_{20}), 168.3 (N–C=O), 141.0 (C_5) and 121.7 (C_6). (Found: C, 72.08; H, 9.16; N, 3.01. Calc. for $C_{25}H_{37}NO_2S$: C, 72.24; H, 8.97; N, 3.37%.)

The filtrate was concentrated to 2 ml to give an additional 0.15 (m.p. 178–181°, 17% yield) of the desired compound as an allotropic modification of the first fraction identical with the first fraction by NMR, IR and tlc.

Preparation of 5-pregnene-20-one-3-spiro-2'-(3'-carbobenzyloxyglycyl-1',3'-thiazolidine) (**7**). 5-Pregnene-20-one-3-spiro-2'-(1',3'-thiazolidine) (1.3 g, 0.00348 mol) was dissolved in 10 ml CH_2Cl_2 and allowed to react with 1.6 g (0.004 mol) carbobenzyloxyglycine anhydride[8] at room temp. for 24 hr. The mixture was diluted with 100 ml of CH_2Cl_2. The CH_2Cl_2 soln was washed with 20 ml water, dried over Na_2SO_4 and concentrated to dryness. The residue was crystallized from 5 ml MeOH to give 0.48 g (m.p. 158–160°, 24% yield) of the desired compound: IR (KBr) 3400 and 3300 cm^{-1} (w) (N–H) and 1725, 1705, 1660 and 1645 (s) (C=O); ^{1}H NMR ($CDCl_3$) δ 7.21 (s, 5, $C_6\underline{H}_5$–), 5.9–5.6 (m, 1, N–$\underline{H}$), 5.5–5.3 (m, 1, C$\underline{H}$=C), 5.13 (s, 2, OC$\underline{H}_2$–C_6H_5), 4.2–3.7 (m, 4, C$\underline{H}_2$–N and N–C$\underline{H}$C=O), 2.9 (t, J=6H$_z$, 2, C$\underline{H}_2$–S), 2.13 (s, 3, $C\underline{H}_3$C=O), 1.11 (s, 3, $C\underline{H}_3$), 0.65 (s, 3, C$\underline{H}_3$–C) and 3.5–1.0 (m, 20, C$\underline{H}_2$ and C$\underline{H}$). (Found: C, 69.91; H, 7.78; N, 4.65. Calc. for $C_{33}H_{44}N_2O_4S$: C, 70.18; H, 7.85; N, 4.96%.)

Preparation of 5-pregnene-20-one-3-spiro-2'-(3'-formyl-1',3'-thiazolidine) (**8**). 5-Pregnene-20-one-3-spiro-2'-(1',3'-thiazolidine) (650 mg) was added to 15 ml of acetic-formic anhydride.[9] The soln was stirred for 2 hr and the solvent evaporated to about 5 ml. The mixture was diluted with 15 ml water, stirred for 30 min and filtered. The solid was dried and recrystalized from EtOAc to

give 276 mg (40% yield) of the desired product as a pale yellow solid: m.p. 192–194°; IR (KBr) 1710 and 1665 cm^{-1} (s) (C=O); ^1H NMR ($CDCl_3$) δ 0.65 (s, 3; CH_3–C), 1.1 (s, 3, CH_3–C), 0.77–3.27 (m, 19, CH_2 and CH), 2.13 (s, 3, CH_3C=O), 2.87 (t, 2, J=6H_z, N–CH_2), 5.5–5.2 (m, 1, CH=C) and 8.40 (s, 1, HC=O). (Found: C, 71.80; H, 8.95; N, 3.15. Calc. for $C_{24}H_{34}NO_2S$: C, 71.95; H, 8.56; N, 3.50%.)

Preparation of 5-androstene-17β-propionate-3-spiro-2'-(3'-acetyl-1',3'-thiazolidine) (**10**). 5-Androstene-17β-propionate-3-spiro-2'-(1',3'-thiazolidine) (0.80 g, 0.002 mol) was dissolved in CH_2Cl_2 (10 ml) and allowed to react with 0.45 g of Ac_2O at room temp. for 24 hr. The reaction was processed as in the previous reactions and crystallized from MeOH to give 0.15 g (m.p. 183–185°, 17% yield) of **10**: IR (KBr) 1720 and 1650 cm^{-1} (s) (C=O); ^1H NMR ($CDCl_3$) δ 5.4–5.1 (m, 1, CH=C), 4.53 (t, 1, J=7H_z, CH–O_2C), 3.83 (t, 2, J=6H_z, CH_2–$NCOCH_3$), 2.72 (t, 2, J=6H_z, CH_2–S), 2.07 (s, 3, CH_3CON), 1.08 (s, 3, CH_3–C), 0.78 (s, 3, CH_3–C). (Found: C, 69.81; H, 8.84; N, 3.08. Calc. for $C_{26}H_{39}NO_3S$: C, 70.08; H, 8.82; N, 3.14%).

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