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STUDIES ON CONFORMATION AND REACTIVITY—V[1]

A NUCLEAR MAGNETIC RESONANCE STUDY OF 4-ETHYLTHIO-4-EN-3-OXO STEROIDS AND THEIR ANALOGS: THE UNIQUE DESHIELDING EFFECT OF A POLAR THIO-FUNCTION

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Abstract—A doublet centered at τ 6·22 in the NMR spectra of 4-ethylthiocholest-4-en-3-one, 17β-acetoxy-4-ethylthioandrost-4-en-3-one, 16α,17α,-epoxy-4-ethylthiopregn-4-ene-3,20-dione, and 17α-hydroxy- and 17α-acetoxy-4-ethylthiopregn-4-ene-3,20-diones, is observed and is attributed to one half of an AB-type quartet (J = 14·5 c/s) resulting from the 6-methylene protons. The unusually large downfield shift of the doublet assigned to the C-6 equatorial proton is presumed to be due to the deshielding effect of the ethylthio function at C-4. The chemical shift of the 6α-proton in cholest-4-en-3-one and its 4-substituted analogs, Me, OH, OMe, OAc, Cl, Br, SH, SAc, S-S-bis and S-bis, are tabulated and the deshielding effect of alkylthio group is found to be more than 1 ppm which is the strongest among these various functions. The unique deshielding effect of alkylthio function is shown to be general by examination of 2-ethylthio-3,5,5-trimethylcyclohex-2-enone and its 2-substituted analogs.

An anomalously weak deshielding effect by the SH group on a double bond is pointed out.

INTRODUCTION

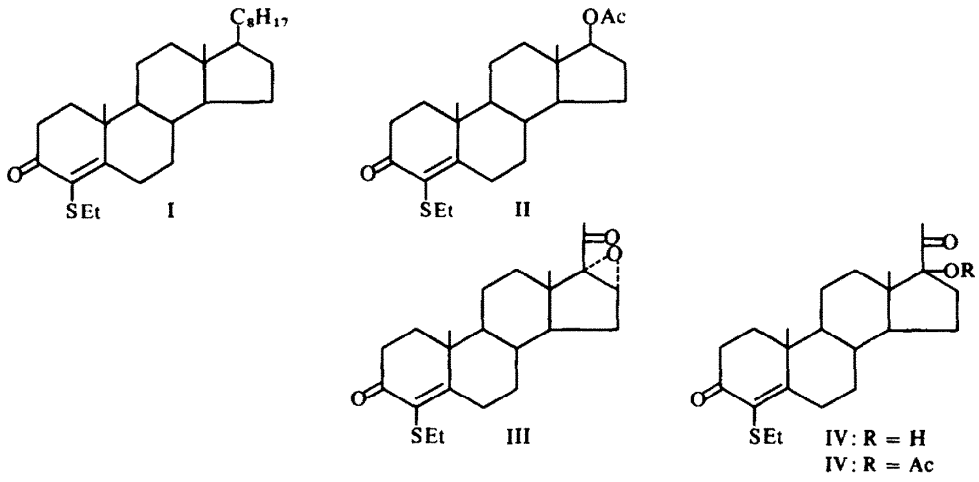
THE analytical application of NMR spectroscopy to the structure and stereochemistry of complex molecules has so far been of great value and interest to organic chemists. In this connection much attention has been focused on the long-range deshielding effect of hetero functions such as nitrogen, halogen or oxygen on protons in their vicinity, and its application to structure elucidation studies.[4]

Several groups have reported[5–13] NMR data on steroids with one or more thio-functions. The present paper deals with an unusually large long-range deshielding effect of a polar thio-function on the 6α-proton in 4-thiosubstituted 4-en-3-oxo steroids, and on the 3-Me group in 2-thiosubstituted 3,5,5-trimethylcyclohex-2-enones. A preliminary communication has already appeared.[14]

NMR SPECTRA OF 4-ETHYLTHIO-4-EN-3-OXO STEROIDS AND THEIR DERIVATIVES: A UNIQUE LONG-RANGE DESHIELDING EFFECT OF THE POLAR THIO-FUNCTION AT C-4 ON THE 6α-HYDROGEN

In the course of a series of our investigations on the polyphosphoric acid (PPA)-catalysed ring opening of 4,5-epoxy-3-oxo steroids and its stereochemistry, we* have achieved a convenient synthesis of 4-ethylthio-4-en-3-oxo systems, i.e. 4-ethylthiocholest-4-en-3-one (I),[15] 17β-acetoxy-4-ethylthioandrost-4-en-3-one (II),[16] 16α,-17α-epoxy-4-ethylthiopregn-4-ene-3,20-dione (III),[17] and 17α-hydroxy- (IV) and

* Three of the present authors (M.T., M.I. and T.F.) and our colleagues.



17α-acetoxy-4-ethylthiopregn-4-ene-3,20-dione (V)[18] by the ring opening of their corresponding 4,5-epoxy-3-oxo systems with ethanethiol.

The NMR spectra (Fig. 1) of these thio-steroids taken at 60 Mc showed doublets

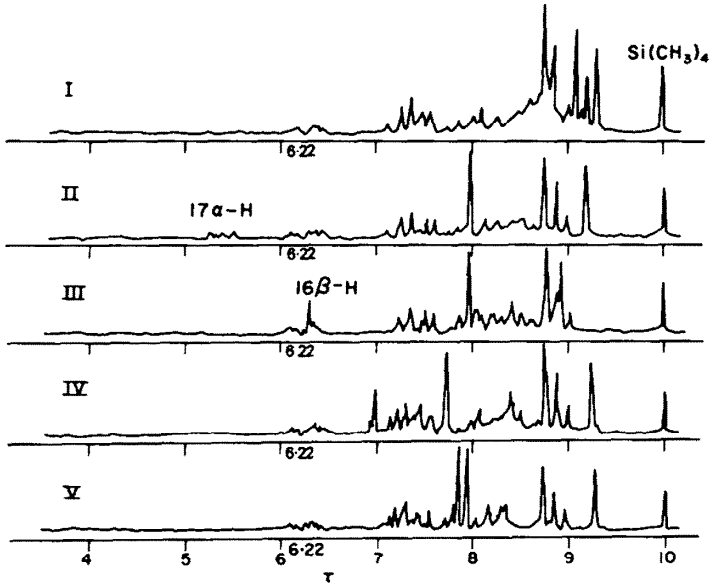


FIG. 1.

(J = 14·5 c/s), centred at τ 6·22.* For instance, the doublet of II is shown in Fig. 2.

The splitting of the signals is due to spin–spin coupling and not to chemical shift since the intensities of the signals are exactly proportional to one proton, and in the spectrum of II taken at 40 Mc† the signal in question also appears as a doublet with almost the same value of J, 14·4 c/s (Fig. 3).

* We have tentatively taken the center of the doublets as the position of the signals. The exact position of the doublet signals as a half of the quartet resulting from the methylene hydrogens at C-6, should be situated upfield from the center. However, its correction was calculated and found to be less than 0·02 ppm with the experimental errors.

† The spectrum was run on a Japan Electronic Optics Laboratory J.N.M. 3 high resolution spectrometer.

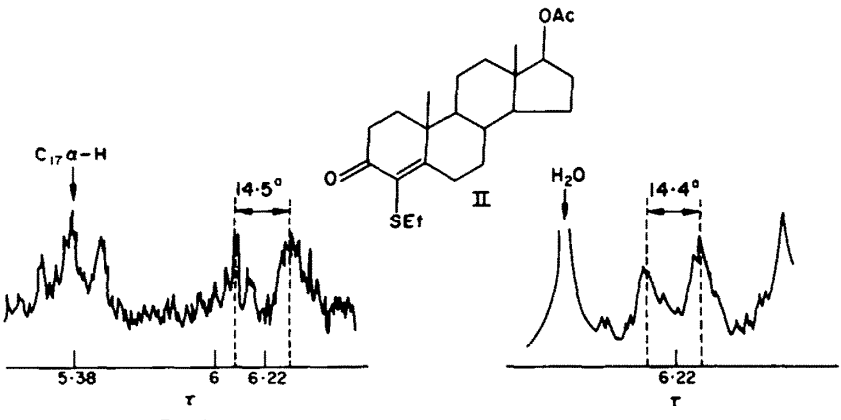
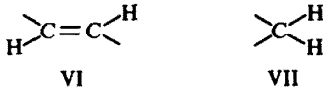
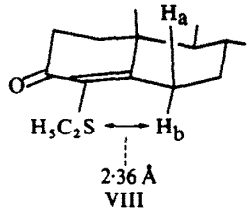


FIG. 2. FIG. 3.

The possibility that a spin–spin coupling constant as large as 14·5 c/s may be characteristic of the structural types VI or VII,[19] together with fact[4] that neighbouring functional groups can cause a downfield shift to a proton resonance, suggested that the signals might originate from a hydrogen of a cyclic methylene group* suffering a spatial interaction with the polar C-4 sulphur function in the compounds I–V.

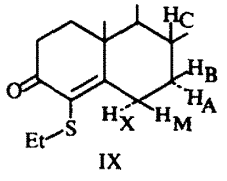


VI VII



VIII

Dreiding models (see VIII) exhibiting the stereochemistry of rings A and B of I–V were therefore examined, and the distance between the non-bonded C_4-sulphur atom and the equatorial $C_6\alpha$-hydrogen, H_b, was calculated to be 2·36 Å† which is even 0·15 Å shorter than the distance between 1,3-diaxial hydrogens in a cyclohexane ring.[21] It thus seemed likely that the close proximity of the 4-S and 6α-H atoms leads to a spatial interaction of some type between these atoms, and that as a result, the 6α-H atom suffers a deshielding effect of the 4-ethylthio group.



IX

Theoretically the 6α-hydrogen may be designated by the letter X relative to the designation of the letters M, A, B, and C to 6α-, 7α-, 7β-, and 8β- protons (see IX). A resulting signal due to H_X in the ABC---MX system could be rather complex.

In fact, the doublet of II (Fig. 2), for instance, does not show a hyperfine structure, and (a) the peaks of the doublets of I–V are broad, (b) the mean widths at half height

* The hydrogens of this type usually give rise to signals higher than τ 8·0.[20]

† The distance between the sulphur atom and the axial 6β-hydrogen atom, H_a, was found to be 3·36 Å.

of the peaks at lower and higher fields of the doublets of I, II, IV and V are 6·6 and 7·5 c/s, respectively,* and (c) the relative intensity in average of these peaks is 1 : 1·5. Meanwhile the observed value of less than 8 c/s for half band widths of each peak of the doublets provided evidence for the equatorial character of the hydrogen responsible for the doublets.†

From the available evidence so far obtained, it was deduced that the doublet signals in question are attributed to one half of a quartet with a J as large as 14·5 c/s resulting from the 6-methylene protons,‡ and that the signals do originate from the 6α-hydrogen under a deshielding effect of the non-bonded 4-ethylthio group.

For further confirmation for the spatial interaction between the 4-sulphur function and 6α-hydrogen atom in the 4-en-3-oxo system, and also for characterization of the nature of the observed deshielding effect of the ethylthio function for the proton resonance, the following 4-substituted 4-en-3-oxo derivatives in the cholestane series were synthesized, and their NMR spectra examined. The compounds prepared for this purpose were cholest-4-en-3-one (X)[23] (the reference compound), 4-methyl- (XII),[24] 4-hydroxy- (XIII),[25] 4-methoxy- (XIV),[26] 4-acetoxy- (XV),[27] 4-chloro- (XVI),[28] 4-bromo- (XVII),[29] 4-*mercapto*- (XVIII) and 4-*acetylthio*- (XIX) *cholest*-4-en-3-*ones*, and *bis*(*cholest*-4-*en*-3-*on*-4-*yl*)*sulphide* (XX) and its *disulphide* (XXI).

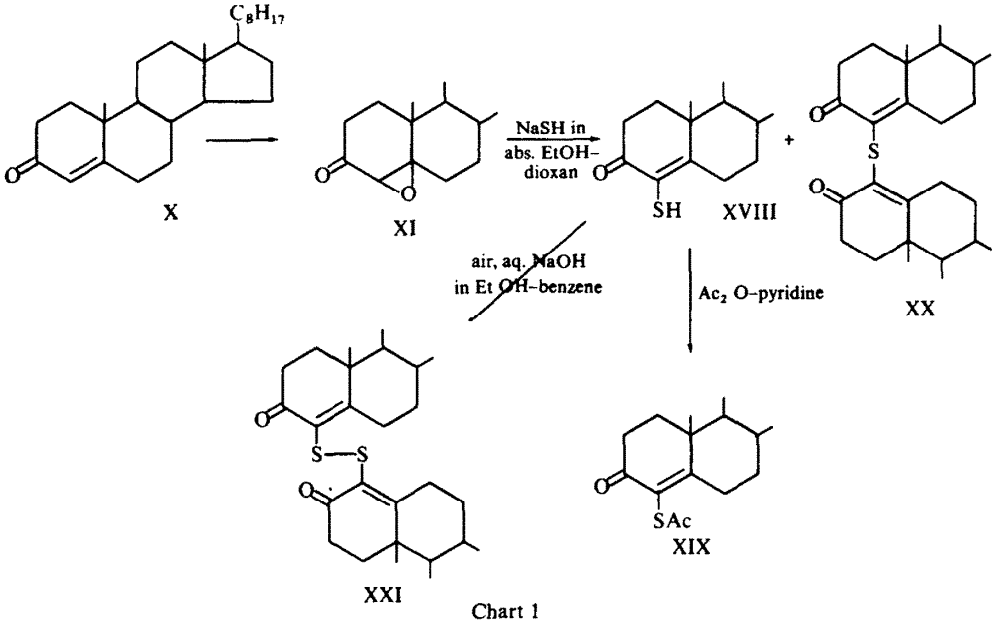


Chart 1

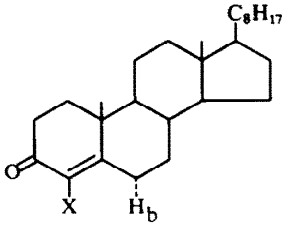
Seven of these compounds, X, XII ~ XVII, are known and were prepared according to the literature. The remaining four thio-steroids, XVIII ~ XXI, were synthesized by the base-catalysed ring opening reaction[30] of 4β,5-epoxy-5β-cholestan-3-one

* The compound III was not used for the calculation as the doublet of III is overlapped by the singlet due to the 16β-hydrogen, and the calculation would therefore be erroneous.

† For coupling with an axial hydrogen, the half-band width of the peaks would be larger than 10 c/s.[22]

‡ The remaining doublet due to the 6β-hydrogen might not be detected as a hidden signal in the methylene envelope of the steroid nucleus above τ 7.

TABLE 1. CHEMICAL SHIFT VALUES OF 6α-H (H_b) OF 4-SUBSTITUTED CHOLEST-4-EN-3-ONES, XII ~ XXI



C_4-Substituents (X)		Signal of C_6α-H (H_b)	Shift value[c], ppm
H	(X)	(≥ 7·58)[b]	—
Me	(XII)	(7·21)[b]	(≥ −0·27)[b]
OH	(XIII)	6·96	≥ −0·62
OMe	(XIV)	6·93	≥ −0·65
OAc	(XV)	(7·28)[b]	(≥ −0·30)[b]
Cl	(XVI)	6·74	≥ −0·84
Br	(XVII)	6·72	≥ −0·86
SH	(XVIII)	(7·22)[b]	(≥ −0·36)[b]
SAc	(XIX)	6·86	≥ −0·72
S—$C_{27}H_{43}O$	(XX)	6·43	≥ −1·15
S—S—$C_{27}H_{43}O$	(XXI)	6·42	≥ −1·16
SEt	(I)	6·22	≥ −1·36

[a] Taken on a Varian A-60 high resolution spectrometer at 60 Mc in $CDCl_3$.

[b] The exact positions are not certain, the signals being masked in the cyclic methylene envelope higher than τ 7·0.

[c] A negative value denotes a downfield shift.

(XI)[31] with sodium hydrogen sulphide followed by treatments such as acetylation or air–oxidation as shown in chart 1.*

As illustrated in Table 1, the NMR spectra of XIII, XIV, XVI, XVII and XIX ~ XXI show similar signals at < τ 7† due to 6α-hydrogen under the deshielding effects

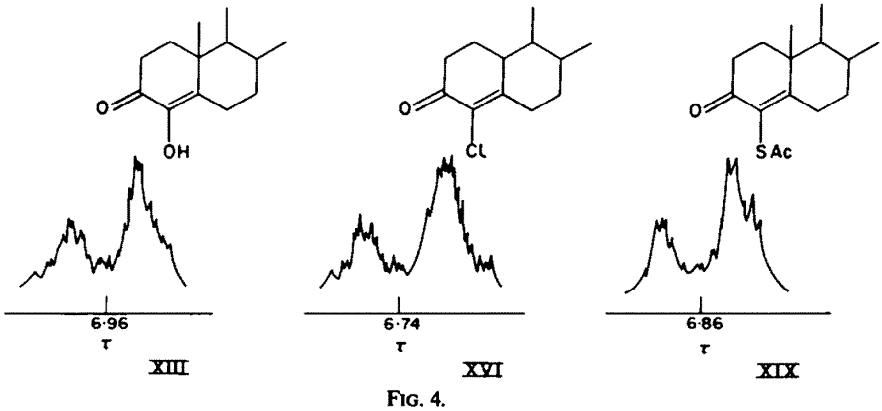


FIG. 4.

* Formation of the sulphide XX was understandable as the result of the reaction of the mercaptan XVIII formed and the remaining starting material XI.

† We could not be certain of the exact positions of signals due to 6α-H appearing higher field than τ 7·0 in the spectra of X, XII, XV, and XVIII, since the signals were almost obscure in the methylene envelope.

of various functional groups at C-4. The intensities of these signals as doublets (J = 14·5 c/s), were proved to correspond to one proton. The fine structure of the signals of, for instance, XIII, XVI and XIX are shown in Fig. 4.

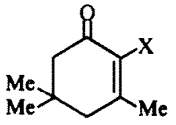
It is therefore concluded that the spatial interaction between a polar electronegative substituent and a proton in a 1,3-"diequatorial" position is to deshield the proton. The order of the deshielding effects of polar functions for the proton resonance observed in 4-substituted 4-en-3-oxo steroids we have so far prepared may therefore be summarized as

$$H < Me, SH < OAc < OH, OMe < SAc < Cl, Br < SR.$$

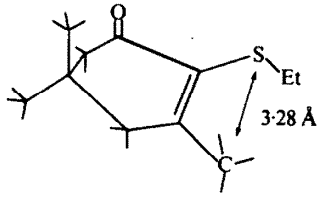
Finally, the effect of the ethylthio group is the strongest, inducing a downfield shift of more than 1 ppm.

NMR SPECTRA OF 2-ETHYLTHIO-3,5,5-TRIMETHYLCYCLOHEX-2-ENONE AND ITS 2-SUBSTITUTED ANALOGS

In an attempt to demonstrate the generality of the deshielding effect of polar thio-functions observed in the steroid system, the 2-substituted 3,5,5-trimethylcyclohex-2-enone system (XXII) was chosen for further investigation. Examination of Dreiding models of a preferred conformation, XXIV, of 2-ethylthio-3,5,5-trimethylcyclohex-2-enone (XXIII), suggested the distance between the sulphur atom at C-2 and Me



XXII



XXIV

group at C-3 to be 3·28 Å.* This was anticipated to be small enough to allow possible occurrence of similar spatial interactions between the two groups.† Furthermore, it was of additional interest to use the cyclohexanone system in which the protons to be examined belong to a freely rotating Me group, whereas the type of protons which has been examined in the steroid system belongs to the rather rigid cyclic methylene group.

The compounds synthesized from 3,5,5-trimethylcyclohex-2-enone (isophorone; XXV) or from its 2,3-epoxide (XXVI) were as follows: 2-*ethylthio*- (XXIII), 2-hydroxy- (XXVII),[33] 2-*acetoxy*- (XXVIII), 2-*chloro*- (XXIX), 2-*mercapto*- (XXX), and 2-*acetylthio*- (XXXI) 3,5,5-*trimethylcyclohex*-2-*enones*, and *bis*(3,5,5-*trimethylcyclohex*-2-*enon*-2-*yl*)*disulphide* (XXXII). The diosphenol (XXVII) is a known compound and was prepared according to the literature. Syntheses of the remaining new compounds were carried out along lines shown in chart 2.

The NMR spectra of XXV (the reference compound), XXIII and XXIX, are shown in Fig. 5, and the relevant data are summarized in Table 2.

From the available data in Table 2, it can be seen that the polar thio-function exhibits a deshielding effect on the Me resonance in the 3,5,5-trimethylcyclohex-2-

* Our thanks are due to Professor T. Yamana and Dr. Y. Mizukami of the Faculty of Pharmaceutical Sciences, Kanazawa University, for their interest and valuable advice in calculation of the distance.

† The van der Waals radii of sulphur and hydrogen atoms are reported to be 1·9 and 1·2 Å respectively.[32]

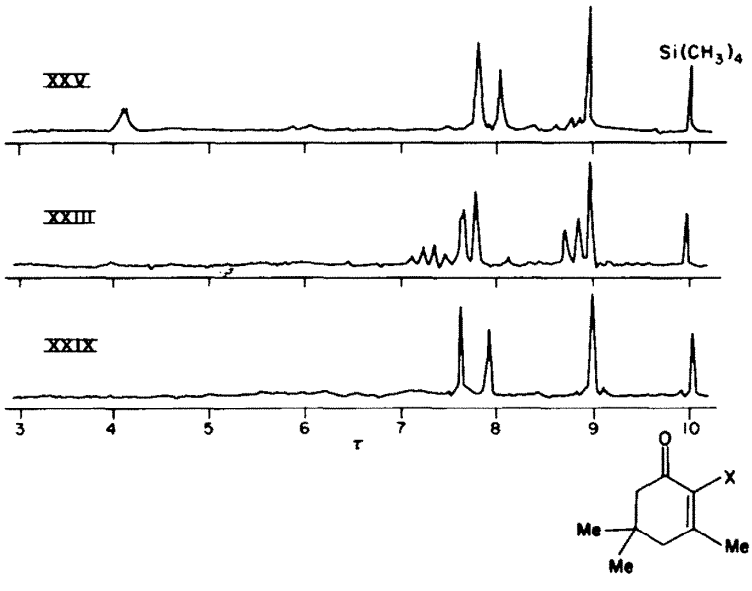
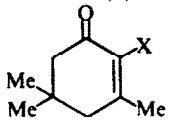


FIG. 5.

TABLE 2. NMR DATA OF 2-SUBSTITUTED 3,5,5-TRIMETHYLCYCLOHEX-2-ENONES



Compound	Substituent X at C-2	NMR data τ					
		C-CH_3 (shift value, ppm)[b]		4-CH_2 and 6-CH_2		5-$(CH_3)_2$	Others
XXV	H	8·03	(—)	7·78		8·96	C_2—H, 4·08[c]
XXVII	OH	8·09[c]	(+0·06)	7·62,	7·70	8·93	C_2—OH, 3·91[e]
XXVIII	OAc	8·15	(+0·12)	7·61		8·88	2-OAc, 7·74
XXIX	Cl	7·87	(−0·16)	7·57		8·92	
XXIII	SEt	7·75	(−0·28)	7·60,[d]	7·62	8·96	S—$\underline{CH_2}$—CH_3, 7·27[f] S—CH_2—$\underline{CH_3}$, 8·84[g]
XXX	SH	7·97	(−0·03)	7·63,	7·68	8·97	2-SH, 5·47
XXXI	SAc	7·88	(−0·15)	7·51,	7·56	8·90	2-SAc, 7·61
XXXII	S—S—$C_9H_{13}O$	7·77	(−0·26)	7·57,	7·62	8·97	

[a] Taken on a Varian A-60 high resolution spectrometer at 60 Mc in $CDCl_3$. Intensities of the signals were proportional to their corresponding proton numbers.

[b] A negative value denotes a downfield shift.

[c] Triplet, J = 1·0 c/s.

[d] Shoulder.

[e] Broad.

[f] Quartet, J = 7·5 c/s.

[g] Triplet, J = 7·5 c/s.

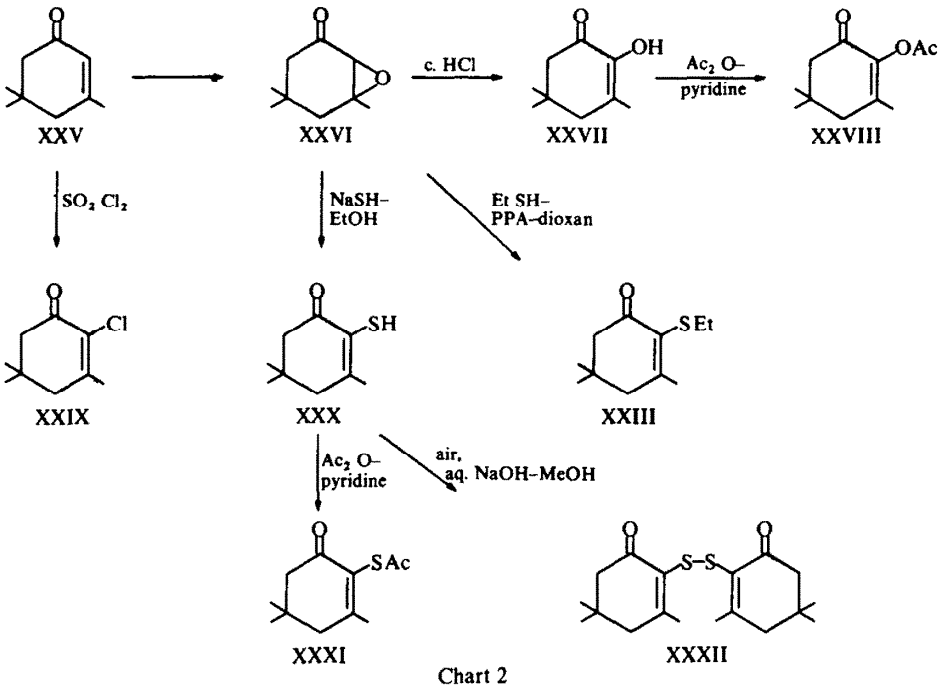
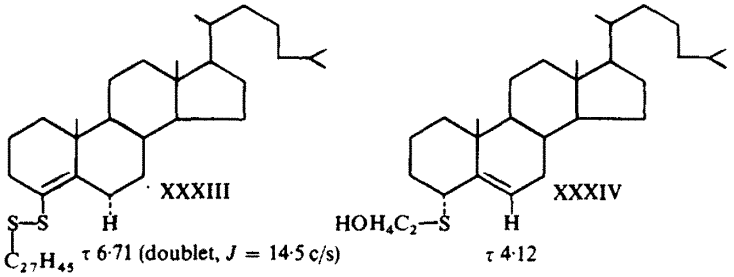


Chart 2

enone system. Comparison of the observed effects of various 2-substituents on the 3-Me group shows that the ethylthio group causes the largest downfield shift of 0·28 ppm, which is a fifth of the shift by the same group in the steroid system.

DISCUSSION

It has recently been shown[34] that *bis-(cholest-4-en-4-yl)disulphide* (XXXIII) and 4α-*(2-hydroxyethylthio)cholest-5-ene* (XXXIV), obtained by lithium–liquid ammonia reduction* of cholesta-3,5-dieno[3.4-b]oxathiane,[15] gives valuable NMR information for the present investigation. The former compound XXXIII with a 4-thio-substituted 4-ene system exhibits a characteristic doublet (J = 14·5 c/s) at τ 6·71 assignable to 6α-proton. The latter compound XXXIV, which has an alternate system of 4-substituted 4-ene systems regarding relative positions of substituent and proton to be examined, exhibits a 6-vinylic proton signal at τ 4·12, which appears 0·70 ppm lower field than the corresponding 6-proton of cholest-5-ene, τ 4·82.[35] These data

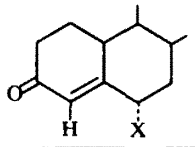


* The reduction has been proved to give, added to XXXIII and XXXIV, cholest-4-ene and [3.4-b]-oxathiano-5α-cholest-3-ene.[34]

provide further support for the suggestion that a polar thio-function exerts a strong deshielding effect on the neighbouring proton conformationally in a 1,3-diequatorial relationship.

Meanwhile, a substantial amount of NMR has been presented[8,36–38] on 6-substituted 4-en-3-oxo steroids, from which we could obtain the following valuable data (Table 3) on additional shifts of 4-hydrogen signals due to 6α-substituents. The data clearly indicate the presence of a spatial interaction between 4-vinylic hydrogen and 6α-substituents in the system, which is another alternate system to the 4-substituted 4-en-3-oxo system we have dealt with. Added to the data in Table 3, a similar interaction between a 6α-substituent (Br) and 4-hydrogen in the 6-substituted 4,5-epoxy system and dealing with NMR data of steroidal 4,5-epoxy derivatives has been reported.[39] All this evidence confirms the deshielding effect, we have observed, by polar electronegative substituents on the proton in a 1,3-diequatorial position with respect to the substituent.

TABLE 3. EFFECTS OF 6α-SUBSTITUENTS UPON 4-VINYLIC PROTONS IN 4-EN-3-OXO STEROIDS[8,36–38,a]



6α-Substituents X	Shift values of 4-H, ppm
(H)	(τ 4·45[b])
Me	−0·10[b]
	−0·05[c]
OH	~ −0·45[d]
	−0·64[b]
OAc	~ −0·13[d]
	−0·16[b]
SAc	~ −0·40[d]
F	~ −0·38[d]
	−0·50[e]
Cl	−0·67[d]
Br	−0·68[d]
	−0·77[c]
NMe_2	−0·48[d]
NHAc	~ −0·11[d]

[a] A negative sign represents a downfield shift.
[b] Ref. 48.
[c] Ref. 49.
[d] Ref. 8.
[e] Ref. 50.

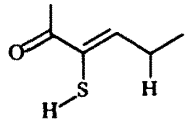
In addition, there are many references, mainly in the steroid field, on the effect of substituents on the proton resonance, for instance, in a 1,3-diaxial position with respect to the substituents. Some of the data[8,11,13,34,36,40–45] are tabulated in Table 4.

TABLE 4. EFFECTS OF SUBSTITUENTS UPON PROTONS OCCUPYING A 1,3-DIAXIAL POSITION[8, 11, 13, 34, 36, 40–45, a] ON THE STEROID NUCLEUS

Substituent X	Chemical shift values, ppm: Relative positions of substituent X and affected proton H)				
	5α-X → 3α-H	2β-H → 19-H	4β-X → 19-H	6β-X → 19-H	11β-X → 19-H
Me				−0·10[b] −0·08[c]	
OH	~ −0·50[d]	−0·25[e] ~ −0·26[d]	−0·27[e]	−0·16[b] ~ −0·20[f] −0·23[d, e]	−0·26[e]
Me				−0·08[b]	
OAc	−0·02[d]	−0·15[e] −0·16[d]	−0·23[e]	−0·10[f] −0·11[b] −0·18[d, e]	−0·07[e]
SH	~ −0·78[d]	−0·28[d]	−0·24[d]	~ −0·27[d]	
SAc	−0·60[d]	−0·13[d]	−0·08[13]	~ −0·10[d, f]	
F		~ −0·16[g]		~ −0·12[f] −0·17[h]	~ −0·30[f] −0·32[e]
Cl	−0·63[d]			~ −0·30[f] −0·32[e]	
Br	−0·72[d]	−0·23[i]		~ −0·25[i] −0·34[b] ~ −0·36[f] ~ −0·37[j]	
CN	~ −0·50[k] ~ −0·57[d]			−0·28[l]	
SCN	−0·63[d]		−0·07[d]	−0·09[d] ~ −0·26[f]	
NMe_2				~ −0·21[f]	
NHAc				~ −0·15[f]	
	(5α, 6α-X) ~ −0·28[d]				
	(5α, 6α-X) ~ −0·24[l]				

[a] A negative value denotes a downfield shift, [b] Ref. 36, [c] Ref. 45, [d] Ref. 13, [e] Ref. 42, [f] Ref. 8, [g] Ref. 40, [h] Ref. 41, [i] Ref. 43, [j] Ref. 34, [k] Ref. 44, [l] Ref. 11.

Examination of all the data presented indicates the chemical shift value of a proton signal due to a substituent under either 1,3-diaxial or 1,3-diequatorial positions, depends upon the structural relationship. Careful consideration should therefore be given before arriving at any conclusion regarding the deshielding effects of polar substituents from given data. We should, however, point out that there seems to be a discrepancy between our results (Tables 1 and 2) and those tabulated in the literature (Tables 3 and 4). As mentioned, although the SH group in both 4-substituted 4-en-3-oxo steroids and 2-substituted 3,5,5-trimethylcyclohex-2-enones, does not cause an effective downfield shift of the neighbouring proton, after acetylation a substantial deshielding effect, even stronger than that by OH group, was noted. This is in marked contrast with a general observation in Tables 3 and 4 that the SH group on a saturated carbon can induce a strong downfield shift similar to that exerted by Cl or Br atoms, and that its acetylation reduces the effect as is the general case in the acetylation of an OH group.[46] We would suggest that the strikingly small deshielding effect of the SH group quoted is unique and may be limited to an SH group on a double bond or one of the thiodiosphenol type in the 2-mercaptosubstituted 2-en-one system (XXXV).



XXXV

With respect to the nature of deshielding effects by the ethylthio group and other electronegative substituents on the neighbouring proton, the evidence so far obtained is insufficient for definite conclusions but it is of interest, however, that the order of atomic polarizability[47] of hetero atoms,

$$F < O < (C) < Cl < Br < S$$

is well in accord with the observed order of deshielding effects of substituents. Furthermore, the distance between the non-bonded 4-S and 6α-H atoms, 2·36 Å, is found to be shorter than the sum of van der Waals radii of S and H atoms, 3·1 Å. It may therefore be assumed that a van der Waals interaction[48] is possibly an important factor in the deshielding effect of substituents, although polar[49] and magnetic anisotropy[50] effects could also be responsible for the deshielding effect in question. The real nature of the deshielding effect is, therefore, still obscure.

Finally, in the 2-substituted 3,5,5-trimethylcyclohex-2-enone system, the signals of 3-vinylic Me groups in the 2-hydroxy (XXVII) and 2-acetoxy (XXVIII) derivatives appear at a higher field than the reference compound XXV without a substituent at C-2. The reason for this anomaly is also obscure.

EXPERIMENTAL

The NMR spectra were taken on a Varian A-60 high resolution spectrometer in $CDCl_3$ containing TMS as internal reference at 27° unless otherwise stated. Chemical shifts are expressed in τ values and coupling constants in c/s. The spectra were analysed generally by the approximation of first order, and there was no ambiguity in the signal assignment.

All the compounds examined in the present investigation were synthesized by the present authors. The m.ps were determined on a Kofler-type hot stage and are uncorrected.

Optical rotations refer to $CHCl_3$, IR spectra to Nujol, and UV spectra to 95% EtOH unless otherwise stated.

4-*Mercaptocholest-4-en-3-one* (XVIII). To a soln of XI (2·0 g) in a mixture of abs EtOH ol (30 ml) and dioxan (30 ml), a soln of excess NaHS in abs EtOH was added and the mixture stirred for 1 hr; the whole procedure was carried out under N_2. A colourless ppt was formed. AcOH (3 ml) was then added and the ppt filtered off. The filtrate was extracted into $CHCl_3$ and the $CHCl_3$ layer washed with sat. $NaHCO_3$ aq and H_2O, and dried (Na_2SO_4). Concentration of the filtrate afforded a yellow oil, (1·53 g). This was chromatographed on 46 g silica gel (Davison Co.); elution with 4:1 pet ether–benzene (270 ml) afforded an unidentified colourless solid, (33 mg). Further elution with pet. ether–benzene (540 ml) afforded XVIII as yellow crystals, m.p. 91–92°, (941 mg, 45·3%). Recrystallization from ether–MeOH gave pale yellow needles, m.p. 94·5–95°. (Found: C, 78·04; H, 10·83; S, 7·65. $C_{27}H_{44}OS$ requires: C, 77·84; H, 10·64; S, 7·69%); $[\alpha]_D^{13}$ + 130° (*c* 0·77); λ_{max} mμ (ε): 300 (7080), end absorption at 220 (5600), one drop of 0·1N NaOH added, 354 (3750), end absorption at 220 (13,600); two drops of 0·1N HCl added to the alkaline soln, 302 (5470), end absorption at 220 (5000); γ_{max} cm^{-1}: 2525 (w) (SH), 1671 (s) (C═O), 1572 (w) (C_4═C_5); NMR τ: 5·31 (1 proton, singlet) (SH), 8·82 (3 protons, singlet) (19-Me), 9·28 (3 protons, singlet) (18-Me).

XX could be obtained from the crude product, the data of which will be described below.

4-*Acetylthiocholest-4-en-3-one* (XIX). Compound XVIII (414 mg) was dissolved in a mixture of pyridine (3 ml) and Ac_2O (3 ml), and the soln kept at room temp for 41 hr when the reaction was complete (TLC); the whole procedure was carried out under N_2. A piece of ice was then added to hydrolyze excess Ac_2O, and the mixture was poured into ice-water depositing a yellow solid, m.p. 95·5–96°, (305 mg). The aqueous filtrate was extracted into ether and the ethereal layer washed with water, and dried (Na_2SO_4). Concentration of the filtrate afforded an oil, (43 mg). The solid and oil were combined, and chromatographed on 10·2 g silica gel (Davison Co.); elution with 19:1 benzene–ether (100 ml) afforded yellow crystals, (318 mg). They were further subjected to TLC on 9 g Kiesel Gel G (Merck Co.) with 19:1 benzene–ether as eluent, affording XIX as yellow crystals, m.p. 95–96°, (245 mg, 53·8%). Recrystallization from MeOH afforded pale yellow prisms, m.p. 98·5–99·5°. (Found: C, 75·93; H, 10·32; S, 6·71. $C_{29}H_{46}O_2S$ requires: C, 75·95; H, 10·11; S, 6·99%); $[\alpha]_D^{13}$ + 116° (*c* 0·72); λ_{max} mμ (ε): 237 (12,200); ν_{max} cm^{-1}: 1698 (s) (SAc), 1680 (s) (C═O), 1561 (m) (C═C); NMR τ: 6·86 (1 proton, doublet, *J* = 14·5 c/s) (6α-H), 7·62 (3 protons, singlet) (S-Ac), 8·70 (3 protons, singlet) (19-Me), 9·28 (3 protons, singlet) (18-Me).

Bis(cholest-4-en-3-on-4-yl)sulphide (XX). To a soln of NaHS (2·0 g) in abs EtOH (30 ml), a soln of the 4β,5-epoxide (2·0 g) (XI) in abs EtOH (50 ml) was added, and the mixture was stirred at room temp overnight; the whole procedure was carried out under N_2. After addition of AcOH (2·0 ml), the colourless ppt was filtered off, (544 mg), and treated with ether leaving an insoluble colourless solid, m.p. 240–246°, (249 mg). 180 mg of this solid was subjected to TLC over 9·0 g Kiesel Gel G (Merck Co.) with 19:1 benzene–AcOEt as eluent, giving XX as colourless crystals, m.p. 252·5–254·5°, (94 mg, 4·5%). The total yield of XX was calculated to be 6·2%. Recrystallization from benzene–AcOEt afforded material, m.p. 254–256°. (Found: C, 80·37; H, 10·97; S, 3·83. $C_{54}H_{86}O_2S$ requires: C, 80·12; H, 10·83; S, 4·01%); $[\alpha]_D^{14}$ +184° (*c* 0·86); $\lambda_{max}^{n\text{-hexane}}$ mμ (ε): 246 (15,400), 312 (7100); ν_{max} cm^{-1}: 1688 (s), 1673 (m) (C═O), 1574 (m), 1564 (m), 1558 (m) (C═C); NMR τ: 6·42 (1 proton, doublet, *J* = 14·5 c/s) (6α-H), 8·80 (3 protons, singlet) (19-Me), 9·28 (3 protons, singlet) (18-Me).

Bis(cholest-4-en-3-on-4-yl)disulphide (XXI). A soln of XVIII (340 mg) in a mixture of abs EtOH (10 ml) and benzene (10 ml) was made alkaline by addition of NaOH aq, and air was passed into the soln for 2 hr. Concentration of the soln followed by addition of MeOH afforded a yellowish ppt which was filtered off, m.p. 193–197°, (150 mg). The solid was reprecipitated from $CHCl_3$–EtOH to give XXI as pale yellow crystals, m.p. 233–234·5°, (100 mg, 29·4%). The compound was again precipitated from the same solvent to give material, m.p. 242–243·5°. (Found: C, 78·14; H, 10·55; S, 7·44. $C_{54}H_{86}O_2S_2$ requires: C, 77·98; H, 10·42; S, 7·71%); $[\alpha]_D^{13}$ +130° (*c* 0·8); $\lambda_{max}^{n\text{-hexane}}$ mμ (ε): 265 (8840); $\nu_{max}^{CHCl_3}$ cm^{-1}: 1668 (s) (C═O), 1542 (m), 1531 (shoulder) (C═C); NMR τ: 6·43 (proton, doublet, *J* = 14·5 c/s) (6α-H), 8·76 (3 protons, singlet) (19-Me), 9·28 (3 protons, singlet) (18-Me).

3,5,5-*Trimethylcyclohex-2-enone* (XXV). Commercially available isophorone was purified by chromatography on basic alumina (Wako Co., grade III) followed by distillation under reduced press, $b.p._{4.5}$ 72°; λ_{max} mμ (ε): 236 (13,300); ν_{max} cm^{-1}: 1670 (s), 1638 (m) (2-en-1-one); NMR τ: 4·08 (1 proton, singlet) (2-H), 7·78 (4 protons, singlet) (4-H and 6-H), 8·03 (3 protons, singlet) (3-Me), 8·96 (6 protons, singlet) (5-Me).

2-Ethylthio-3,5,5-trimethylcyclohex-2-enone (XXIII). A soln of XXVI (4·0 g), ethanethiol (16 ml) and PPA (8 g) in dioxan (80 ml) was kept at room temp for a week when the reaction was complete (TLC). The reaction mixture was poured into ice-water, depositing a yellow oil. The oil was extracted into ether and the ethereal layer was washed with sat $NaHCO_3$ aq, and water, and dried (Na_2SO_4). Concentration of the filtrate afforded a yellow oil, (5·90 g). This was chromatographed on 180 g basic alumina (Wako Co., grade III); elution with pet. ether (3350 ml) afforded a yellow oil, (2·89 g). which, gradually crystallized and recrystallized from ether to give XXIII as pale yellow needles, m.p. 34–37·5°, (440 mg, 8·6%). Further recrystallization from pet. ether gave colourless needles, m.p. 36·5–38°. (Found: C, 66·76; H, 9·32; S, 15·93. $C_{11}H_{18}OS$ requires: C, 66·61; H, 9·14; S, 16·16%); λ_{max} mμ (ε): 238–242 (9300), 306–310 (2800); $\nu_{max}^{CHCl_3}$ cm^{-1}: 1671 (s) (C=O), 1589 (m) (C=C); NMR τ: 7·28 (2 protons, quartet, J = 7·5 c/s) (S-$\underline{CH_2}$-Me), 7·60 (shoulder) and 7·62 (4 protons, singlet) (4-H and 6-H), 7·75 (3 protons, singlet) (3-Me), 8·87 (3 protons, triplet, J = 7·5 c/s) (S—$\underline{CH_2}$—$\underline{Me}$), 8·96 (6 protons, singlet) (5-Me).

Further elution with 4:1 pet. ether–benzene (350 ml), 1:1 pet. ether–benzene (400 ml) and benzene (400 ml) afforded 244 mg, 538 mg, and 110 mg respectively of yellow oil. Identification of the oils was not carried out.

Desulphurization of XXIII *with Raney nickel: Formation of* XXV. To a soln of XXIII (200 mg) in acetone (30 ml), deactivated Raney Ni (W-2, ca. 2 g) was added and the mixture was refluxed for 9·5 hr and more Raney Ni (ca. 1 g) was added. The mixture was refluxed for further 3·5 hr and the Raney Ni was filtered off. Concentration of the filtrate afforded XXV as a pale yellow oil, (113 mg), λ_{max} mμ (ε): 236 (10,760); ν_{max} cm^{-1}: 1670 (s) (C=O), 1640 (m) (C=C). The oil was distilled under reduced press to give a colourless oil, n_D^{20} 1·4740. The UV and IR spectra were superposable with those of a specimen of XXV.

2-Hydroxy-3.5.5-trimethylcyclohex-2-enone (XXVII). This was prepared from XXVI[33] as colourless needles, m.p. 89–91° (lit. 91–92°); λ_{max} mμ (ε): 275 (9500), made alkaline with one drop of 0·1N NaOH, 276–280 (5700), 312–320 (shoulder, 3600), made acid by addition of two drops of 0·1N HCl to the alkaline soln, 275 (8600): ν_{max} cm^{-1}: 3387 (s) (OH), 1665 (s), 1640 (s) (C=O, C=C); NMR τ: 7·62 and 7·70 (4 protons, singlets) (4-H and 6-H), 8·09 (3 protons, triplet, J = 1·0 c/s) (3-Me), 8·93 (6 protons, singlet) (5-Me).

2-Acetoxy-3,5,5-trimethylcyclohex-2-enone (XXVIII). To a soln of XXVII (1·0 g) in pyridine (5 ml), Ac_2O (5 ml) was added portionwise under ice-cooling, and the mixture was kept under cooled for 44 hr when the reaction was complete (TLC). The mixture was poured into ice-water and then acidified with 10% H_2SO_4, and extracted into ether. The ethereal layer was washed with sat. $NaHCO_3$ aq and water, and dried (Na_2SO_4). Concentration of the filtrate afforded pale yellow crystals. Recrystallization from pet. ether gave XXVIII as colourless leaflets, m.p. 60–62°, (700 mg, 55·2%). (Found: C, 67·68; H, 8·46. $C_{11}H_{16}O_3$ requires: C, 67·32; H, 8·22%); λ_{max} mμ (ε): 242 (10,750); ν_{max} cm^{-1}: 1754 (s) (OAc), 1682 (s) (C=O), 1657 (m), 1640 (shoulder) (C=C); NMR τ: 7·61 (4 protons, singlet) (4-H and 6-H), 7·94 (3 protons, singlet) (MeCOO), 8·15 (three protons, singlet) (3-Me), 8·88 (6 protons, singlet) (5-Me).

2-Chloro-3,5,5-trimethylcyclohex-2-enone (XXIX). To a soln of XXV (10·0 g) in pyridine (50 ml), SO_2Cl_2 (30 ml)[51] was added portionwise in 1 hr at 24–27° and the mixture was kept at the same temp for another hr under stirring; the colour of the mixture changed from yellow to brown black. The mixture was poured into ice–water depositing a brown solid which was filtered off. The filtrate was extracted into ether and the ethereal layer was washed with water, and dried (Na_2SO_4). The filtrate was concentrated to give an oil which was subjected to fractional distillation under reduced press, to give 3 fractions: (a) colourless oil, b.p. $_{4}$63–70°, (0·9 g); (b) colourless oil, b.p. $_{4}$114–119°, (4·7 g) and (c) colourless oil, b.p. $_{4}$123–124°, (0·7 g). Investigation of the most volatile fraction (a) was not performed.

The oils from (b) and (c) fractions were subjected to chromatography on 168 g basic alumina (Wako Co.) elution with pet. ether (780 ml) gave a colourless oil, (1·10 g). The oil was distilled under reduced press to give crude XXIX as a colourless oil, b.p. $_{4}$105–106°. TLC of the oil on Kiesel Gel G (Merck Co.) followed by repeated distillation under reduced press gave the pure sample of XXIX, 300 mg, 2·4%) for the micro- and spectral analyses. (Found: C, 62·15; H, 7·48; Cl, 20·10. $C_9H_{13}OCl$ requires: C, 62·57; H, 7·59; Cl, 20·57%). n_D^{20} 1·4983; λ_{max} mμ (ε): 253 (9900); ν_{max} cm^{-1}: 1690 (s) (C=O), 1618 (m) (C=C); NMR τ: 7·57 (4 protons, singlet) (4-H and 6-H), 7·87 (3 protons, singlet) (3-Me), 8·92 (6 protons, singlet) (5-Me).

Further elution with pet. ether (2640 ml) gave another colourless oil, (1·8 g); further investigation of this oil was not performed.

2-Mercapto-3,5,5-trimethylcyclohex-2-enone (XXX) *and bis(3,5,5-trimethylcyclohex-2-enon-2-yl)disulphide* (XXXII). To a soln of XXVI (14·0 g) in abs EtOH (420 ml), a soln of NaHS (28 g) in abs EtOH (300 ml) was added portionwise with stirring in 5 min at room temp, and the reaction mixture was kept at room

temp for 40 min; the whole procedure was carried out under N_2. After AcOH (5 ml) was added, the mixture was poured into ice–water depositing red crystals, which were recrystallized from MeOH affording red prisms, m.p. 174–178°, (2·597 g). Repeated recrystallization from the same solvent afforded material, m.p. 182–183°. (Found: C, 74·58; H, 8·18; S, 10·17 and 10·68%); λ_{max} mμ (ε): 262 (E, 1%, 1 cm, 668), 488 (E, 1%, 1 cm, 131); ν_{max} cm^{-1}: 1652 (s), 1614 (w), 1571 (w), 1531 (m); NMR τ: 4·49 (singlet), 7·66 (singlet) 7·73 (singlet), 7·84 (doublet, J = 1 c/s), 7·95 (doublet, J = 1 c/s), 8·31 (singlet), 8·93 (singlet), 9·06 (singlet). The structure of the red prisms has not been elucidated.

The aqueous filtrate from the red crystals was extracted into $CHCl_3$, and the $CHCl_3$ layer washed with water and dried, (Na_2SO_4). The procedure was carried out under N_2. Concentration of the $CHCl_3$ filtrate afforded a red oil, (10·0 g), which was chromatographed on 300 g silica gel (Wako Co.); elution with benzene (5 l.) afforded an orange yellow oil, (938 mg), having an absorption max at 294 mμ in its UV spectrum. The oil was purified by fractional distillation under reduced press and under N_2 giving XXX as a pale yellow oil, b.p. $_3$75–80°, (506 mg, 3·3%). Repeated distillation under reduced press and under N_2 gave the compound as a colourless oil, b.p. $_2$70–72°. (Found: C, 63·28; H, 8·19; S, 18·66. $C_9H_{14}OS$ requires: C, 63·48; H, 8·28; S, 18·83%); n_D^{20} 1·5269; λ_{max} mμ (ε): 295 (5780), made alkaline with one drop of 0·1N NaOH, 346 (2500), made acid by addition of two drops of 0·1N HCl to the alkaline soln mentioned above, 295 (3800); ν_{max} cm^{-1}: 2535 (w) (SH), 1669 (s) (C═O), 1608 (m) (C═C); NMR τ: 5·47 (proton, singlet) (SH) 7·63 and 7·68 (4 protons, singlets) (4-H and 6-H), 7·97 (3 protons, singlet) (3-Me), 8·97 (6 protons, singlet) (5-Me).

Further elution with 19:1 benzene–ether (500 ml) afforded an unidentified orange yellow oil, (49 mg). Continuous elution with 1:1 benzene–ether (1000 ml) afforded a brown oil, (8·02 g), which gradually crystallized. The compound was recrystallized from MeOH to give XXXII as pale brown needles, m.p. 158–161°, (412 mg, 5·4%). Further recrystallization from the same solvent afforded colourless needles, m.p. 159–161°. (Found: C, 63·79; H, 7·85; S, 18·29. $C_{18}H_{26}O_2S_2$ requires: C, 63·86; H, 7·74; S, 18·91%); λ_{max} mμ (ε): 244–250 (14,530); ν_{max} cm^{-1}: 1675 (s) (C═O), 1582 (m) (C═C); NMR τ: 7·57 and 7·62 (4 protons, singlets) (4-H and 6-H), 7·77 (3 protons, singlet) (3-Me), 8·97 (6 protons, singlet) (5-Me).

Further elution with ether (500 ml) and MeOH (500 ml) afforded 385 mg and 393 mg respectively of unidentified brown oils.

2-Acetylthio-3,5,5-trimethylcyclohex-2-enone (XXXI). A soln of XXX (850 mg) in a mixture of Ac_2O (3·5 ml) and pyridine (3·5 ml) was kept at room temp overnight and worked up to give a pale yellow oil, (522 mg). This was chromatographed on 15·7 g silica gel (Davison Co.); elution with 19:1 benzene–ether (300 ml) afforded a pale yellow oil, (338 mg), which crystallized. Recrystallization from pet. ether afforded XXXI as colourless needles, m.p. 41–42°, (107 mg, 7·7%). (Found: C, 62·46; H, 7·90; S, 14·36. $C_{11}H_{16}O_2S$ requires: C, 62·22; H, 7·59; S, 15·10%); λ_{max} mμ (ε): 229 (9860); ν_{max} cm^{-1}: 1704 (s), 1693 (s) (SAc), 1673 (s) (C═O), 1596 (m) (C═C); NMR τ: 7·51 and 7·56 (4 protons, singlet) (4-H and 6-H), 7·61 (3 protons, singlet) (SAc), 7·88 (3 protons, singlet) (3-Me), 8·90 (6 protons, singlet) (5-Me).

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REFERENCES

[1] Part IV: M. Tomoeda, A. Ishida and T. Koga, *Chem. Pharm. Bull.* **15**, 887 (1967).
[2] Present address: Research Laboratories, Yoshitomi Pharmaceutical Co., Tokyo.
[3] Present address: Research Laboratories, Shionogi and Co., Osaka.
[4] For instance, see N. S. Bhacca and D. H. Williams, *Applications of NMR Spectroscopy in Organic Chemistry* p. 19. Holden-Day, San Francisco (1964), and Refs cited therein.
[5] L. F. Fieser, C. Yuan and T. Goto, *J. Am. Chem. Soc.* **82**, 1996 (1960).
[6] K. Takeda and T. Komeno, *Chem. Pharm. Bull.* **8**, 672 (1960).
[7] R. C. Tweit: *J. Org. Chem.* **27**, 2693 (1962).
[8] K. Tori and K. Kuriyama, *Chem. & Ind.* 1522 (1963).

[9] K. Takeda and T. Komeno, *Chem. & Ind.* 1793 (1963).
[10] K. Takeda, T. Komeno, J. Kawanami, S. Ishihama, H. Kadokawa, H. Tokura and H. Itani, *Tetrahedron* **21**, 295 (1964).
[11] K. Tori, T. Komeno and T. Nakagawa, *J. Org. Chem.* **29**, 1136 (1964).
[12] C. W. Shoppee, M. I. Akhtar and R. E. Lack, *J. Chem. Soc.* 877 (1964).
[13] K. Tori and T. Komeno, *Tetrahedron* **21**, 309 (1965).
[14] M. Tomoeda, M. Inuzuka, T. Furuta and T. Takahashi, *Tetrahedron Letters* 1233 (1964).
[15] M. Tomoeda, M. Ishizaki, H. Kobayashi, S. Kanatomo, T. Koga, M. Inuzuka and T. Furuta, *Tetrahedron* **21**, 733 (1965).
[16] M. Tomoeda, M. Inuzuka, T. Furuta and T. Koga, *Chem. Pharm. Bull.* **13**, 769 (1965).
[17] M. Tomoeda, T. Furuta and T. Koga, *Chem. Pharm. Bull.* **13**, 1078 (1965).
[18] Ref. cited in (1).
[19] L. M. Jackman, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry* p. 85. Pergamon Press, London (1959).
[20] R. M. Silverstein and G. C. Bassler, *Spectrometric Identification of Organic Compounds* p. 82. Wiley, New York (1963).
[21] O. Hassel, *Quart. Rev.* **7**, 221 (1953).
[22] Ref. 20, p. 86.
[23] J. E. Eastham and R. Teranishi, *Org. Syn.* **35**, 39 (1955).
[24] H. J. Ringold and S. K. Malhotra, *J. Am. Chem. Soc.* **84**, 3402 (1962).
[25] D. J. Collins, *J. Chem. Soc.* 3919 (1959).
[26] W. Reusch and R. LeMahieu, *J. Am. Chem. Soc.* **85**, 1669 (1963).
[27] L. F. Fieser and R. Stevenson, *J. Am. Chem. Soc.* **76**, 1728 (1954).
[28] J. I. Shaw and R. Stevenson: *J. Chem. Soc.* 3549 (1955).
[29] B. Camerino, B. Patelli, A. Vercellone and F. Media: *Il. Farmaco Pavia (Ed. Sci.)* **11**, 586 (1956).
[30] J. M. Krämer, K. Brüchner, K. Irmscher and Karl-Heinz Bork, *Chem. Ber.* **96**, 2803 (1963).
[31] P. A. Plattner, H. Heusser and A. B. Kulkarni, *Helv. Chim. Acta* **31**, 1822 (1948).
[32] E. S. Gould, *Mechanism and Structure in Organic Chemistry* p. 51. Holt, New York (1959).
[33] G. B. Payne, *J. Org. Chem.* **24**, 719 (1959).
[34] M. Tomoeda, A. Ishida, Y. Hiyoshi and T. Koga, *23rd Annual Meeting of the Pharmaceutical Society of Japan* at Sendai, Japan, 22nd October (1966).
[35] G. M. Cragg, C. W. Davey, D. N. Hall, G. D. Meakins, (Mrs.) E. E. Richards and T. L. Wateley: *J. Chem. Soc.* C-**14**, 1266 (1966).
[36] D. J. Collins, J. J. Hobbs and S. Sternhell: *Tetrahedron Letters* 197 (1963).
[37] T. A. Wittstruck, S. K. Malhotra and H. J. Ringold. *J. Am. Chem. Soc.* **85**, 1699 (1963).
[38] T. A. Wittstruck, S. K. Malhotra, H. J. Ringold and A. D. Cross, *J. Am. Chem. Soc.* **85**, 3038 (1963).
[39] D. J. Collins, J. J. Hobbs and S. Sternhell: *Tetrahedron Letters* 623 (1963).
[40] A. D. Cross and P. W. Landis, *J. Am. Chem. Soc.* **86**, 4006 (1964).
[41] A. D. Cross, *J. Am. Chem. Soc.* **86**, 4012 (1964).
[42] R. F. Zürcher, *Helv. Chim. Acta* **44**, 1380 (1961); *J. Am. Chem. Soc.* **46**, 2054 (1963); Ref. 4.
[43] J. C. Jacquesy, J. M. Lehn and J. Levisalles, *Bull. Soc. Chim. Fr.* 2444 (1961).
[44] A. D. Cross and I. T. Harrison: *J. Am. Chem. Soc.* **85**, 3223 (1963).
[45] G. Slomp and B. R. McGarvey, *J. Am. Chem. Soc.* **81**, 2200 (1959).
[46] Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto and K. Tsuda, *Chem. Pharm. Bull.* **10**, 338 (1962).
[47] C. K. Ingold, *Structure and Mechanism in Organic Chemistry* p. 119. Cornell University Press, Ithaca (1953).
[48] See S. Winstein, P. Cater, F. A. L. Anet and A. J. R. Bourn, *J. Am. Chem. Soc.* **87**, 5247 (1965), and Refs cited therein; Ref. 13, p. 318.
[49] A. D. Buckingham, *Canad. J. Chem.* **38**, 300 (1960); W. T. Raynes, A. D. Buckingham and H. J. Bernstein, *J. Chem. Phys.* **36**, 348 (1962); R. F. Zürcher, *NMR in Chemistry* p. 45. Academic Press, New York (1964).
[50] Ref. 19, p. 112; Ref. 4, p. 24.
[51] H. Mori, *Chem. Pharm. Bull.* **10**, 429 (1962); *Farm. Italia Soc. Anon.*; *Brit. Pat.* 874, 572 (1961).