Steroids and Related Studies. Part XXVII.[1] Tetrazoles from Progesterone and 7-Oxocholest-5-en-3β-yl Acetate

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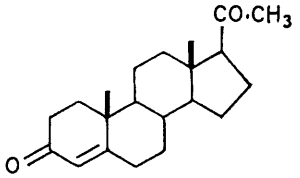
Progesterone when treated with an excess of hydrazoic acid in the presence of boron trifluoride gives a complex mixture from which two pure components have been separated and shown to be 17β-acetamido-3-aza-A-homoandrost-4a-eno[3,4-*d*]tetrazole (2) and 17β-(5-methyltetrazol-1-yl)-3-aza-A-homoandrost-4a-eno[3,4-*d*]tetrazole (4). The u.v., i.r., and n.m.r. spectra corresponding to the 3-aza-A-homo-4a-eno[3,4-*d*]tetrazole system of several steroid analogues have been examined. 7-Oxocholest-5-en-3β-yl acetate yielded 7a-aza-B-homocholest-5-eno[7a,7-*d*]tetrazol-3β-yl acetate (8). Hydrolysis of the ester function of the latter proceeds with allylic shift in basic medium, whereas acid hydrolysis does not show any such change. On Oppenauer oxidation both hydrolysis products give 7a-aza-B-homocholest-4-eno[7a,7-*d*]tetrazol-3-one.

WE have previously synthesised steroidal tetrazoles [2-4] starting with certain 3-oxo-4-eno-steroids, and the studies have been extended using other αβ-unsaturated steroidal ketones. This paper describes the preparation of the tetrazole analogues from progesterone and 7-oxocholest-5-en-3β-yl acetate. In addition, spectral studies are reported for some known 3-aza-A-homo-4a-eno[3,4-*d*]tetrazole steroid derivatives.

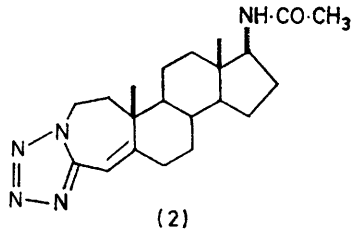
Treatment of progesterone (1) with an excess of hydrazoic acid in the presence of boron trifluoride as a catalyst



(1)

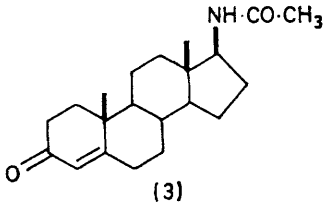
in chloroform yielded a solid residue which was a complex mixture (t.l.c.). Repeated crystallisation from acetone afforded a pure compound, $C_{21}H_{31}N_5O$, m.p. 312—314° (decomp.). The residue from the combined mother liquors on column chromatography gave one other product, $C_{21}H_{30}N_8$, m.p. 275—278°. The elemental compositions of the two compounds were supported by the respective molecular ion peaks in the mass spectra.

The elemental composition of the higher melting product indicated that one of the two carbonyl groups had reacted to form a tetrazole, and an i.r. band at 1670 cm^{-1} was characteristic of an amide or lactam carbonyl stretching, with N–H stretching at 3335 cm^{-1}. The n.m.r. signal at δ 1·97 (3H, s, $COCH_3$) indicated that the 20-oxo-group had reacted to form an acetamido-group, and this was also apparent from the one-proton multiplet at δ 3·92 (17α-H).[5] In light of the earlier work,[2] the two-proton multiplet at δ 4·48 provided conclusive evidence for the presence of 3-aza-A-homo-4a-eno[3,4-*d*]tetrazole system, and the spectral studies thus supported assignment of structure (2) to the product. Earlier, Doorenbos and Singh [6] had shown that progesterone when treated with an equimolar quantity of sodium azide in polyphosphoric acid gave 17β-acetamidoandrost-4-en-3-one (3) showing the 20-oxo-group to be more reactive than the 4-en-3-one function under



(2)

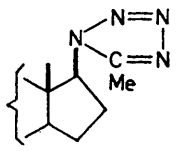
the reaction conditions, whereas use of an excess of sodium azide gave 17β-acetamido-3-aza-A-homoandrost-4a-en-4-one. To further prove structure (2), an authentic specimen of (3) was prepared, and the latter when



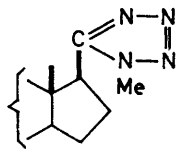
(3)

treated with hydrazoic acid–boron trifluoride in chloroform yielded a product which was identical with the compound obtained above.

The second product showed a two-proton multiplet at δ 4·50 and other spectral evidence also indicated a tetrazole fused to ring A as in (2) but the elemental composition showed that there was also a tetrazole system attached at position 17. The alternative structures for the tetrazole system attached to position 17 are (A) and



(A)

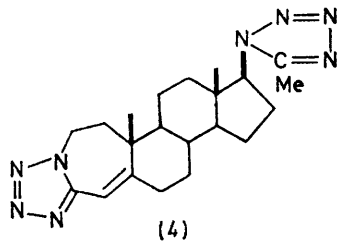


(B)

(B). Mechoulam [7] proposed that formation of (A) from 20-oxo-steroids might be preferred, but no evidence was provided. Our compound showed a signal at δ 2·54

[1] Part XXVI, A. Gandiha, I. G. Marshall, D. Paul, and H. Singh, *J. Pharm. Pharmacol.*, in the press.
[2] H. Singh, R. B. Mathur, and P. P. Sharma, *J.C.S. Perkin I*, 1972, 990.
[3] H. Singh, V. V. Parashar, and R. B. Mathur, *Indian J. Chem.*, 1972, **10**, 241.
[4] H. Singh, R. K. Malhotra, and V. V. Parashar, *Tetrahedron Letters*, 1973, 2587.
[5] C. H. Robinson, C. Ermann, and D. P. Hollis, *Steroids*, 1965, **6**, 509.
[6] N. J. Doorenbos and H. Singh, *J. Pharm. Sci.*, 1962, **51**, 418.
[7] R. Mechoulam, *Israel J. Chem.*, 1968, **6**, 909.

(3H, s) for the methyl attached to the carbon of the tetrazole nucleus, which supports skeleton (A), since the *N*-methyl signal will occur relatively downfield. Tetrazole derivatives are reported to exhibit n.m.r. signals [8] for *C*-methyl and *N*-methyl groups at δ 2·53 and 4·18, respectively. The bistetrazolo-steroid obtained could hence be assigned structure (4). The configuration at



(4)

C-17 would be expected to remain unchanged in the Schmidt reaction.

It may be worthwhile to summarise the pertinent features of the spectra which we have determined of the new and some of the known 3-aza-A-homo-4a-eno-[3,4-*d*]tetrazole steroids prepared in our laboratory (Table). Consistently the two-proton multiplet for the 2-methylene group appeared at *ca.* δ 4·50. Further, the C-4a vinylic proton gave a signal near δ 6·5, which is characteristically downfield as compared with the average position at δ 5·75 of the C-4 proton resonance in 4-en-3-one steroids.[9] It was seen that the 10-methyl singlet moved downfield slightly in 3-aza-A-homo-4a-eno[3,4-*d*]tetrazoles as compared with the signal of the angular methyl group of 4-en-3-ones. For instance, there is a shift of 0·07 p.p.m. in the steroidal tetrazoles (5) and (6) as compared with C-10 methyl singlet at δ 1·20 of the parent cholest-4-en-3-one and (25*R*)-spirost-4-en-3-one, respectively. In the i.r. spectra C=C stretching was shown by a relatively sharp band near 1650 cm^{-1}, and the other significant bands between 1540 and 1375 cm^{-1} may be due to C=N and N=N stretching modes of the tetrazole system. Absorption in the u.v. spectra appeared between 240 and 243 nm, which is close to that in 4-en-3-one steroids; the extension of conjugation in the 3-aza-A-homo-4a-eno[3,4-*d*]tetrazole chromophore does not apparently have much effect on the u.v. absorption.

Spectral data of some compounds containing the 3-aza-A-homo-4a-eno[3,4-*d*]tetrazole system

Compound	N.m.r. (60 Hz; δ) C(2)H_2(m)	C(4a)H	C(19)H_3 (s)	I.r. $\nu_{max.}$(KBr)/cm^{-1}	U.v. $\lambda_{max.}$(EtOH)/nm (log ε)
3-Aza-A-homocholest-4a-eno[3,4-*d*]tetrazole (5) [7]	4·52	6·53	1·27	1661, 1538, 1470, 1380	242 (4·22)
(25*R*)-3-Aza-A-homospirost-4a-eno[3,4-*d*]tetrazole (6) [2]	4·50	6·49	1·27	1650, 1530, 1450, 1380	243 (4·23)
3-Aza-A-homopregna-4a,16-dieno[3,4-*d*]tetrazol-20-one [2]	4·50	6·50	1·28	1660, 1520, 1445, 1375	241 (4·41)
3-Aza-A-homopregna-4a,16-dieno[3,4-*d*]tetrazol-20-one oxime [2]	4·51	6·51	1·28	1648, 1535, 1445, 1375	240 (4·17)
3-Aza-A-homoandrost-4a-eno[3,4-*d*]tetrazol-17-one [2]	4·50	6·50	1·28	1650, 1530, 1445, 1385	242 (4·23)
3,17a-Diaza-A,D-bishomoandrost-4a-eno[3,4-*d*]-tetrazol-17-one [3]	4·50	6·50	1·25	1650, 1530, 1450, 1385	240 (4·21)
3-Aza-A-homoandrost-4a-eno[3,4-*d*]tetrazol-17β-yl acetate [10]	4·50	6·50	1·26	1650, 1530, 1450, 1380	242 (4·23)
17β-Acetamido-3-aza-A-homoandrost-4a-eno[3,4-*d*] tetrazole (2)	4·48	6·50	1·24	1650, 1530, 1450, 1375	243 (4·22)
17β-(5-methyltetrazol-1-yl)-3-aza-A-homoandrost-4a-eno[3,4-*d*]tetrazole (4)	4·50	6·51	1·27	1650, 1520, 1450, 1390	243 (4·23)

The studies were extended to the 7-oxo-5-eno-steroid system, and 7-oxocholest-5-en-3β-yl acetate (7) was selected for exploratory studies. Barnes *et al.*[11] treated 7,11-dioxolanost-8-en-3-yl acetate with hydrazoic acid (H_2SO_4, $CHCl_3$, 0°) and obtained, in addition to two monolactams, a tetrazole which was considered to have been formed by reaction with the 7-oxo-function. Apart from this example, there do not appear to be any other instances of the preparation of ring B fused steroid or triterpenoid tetrazoles.

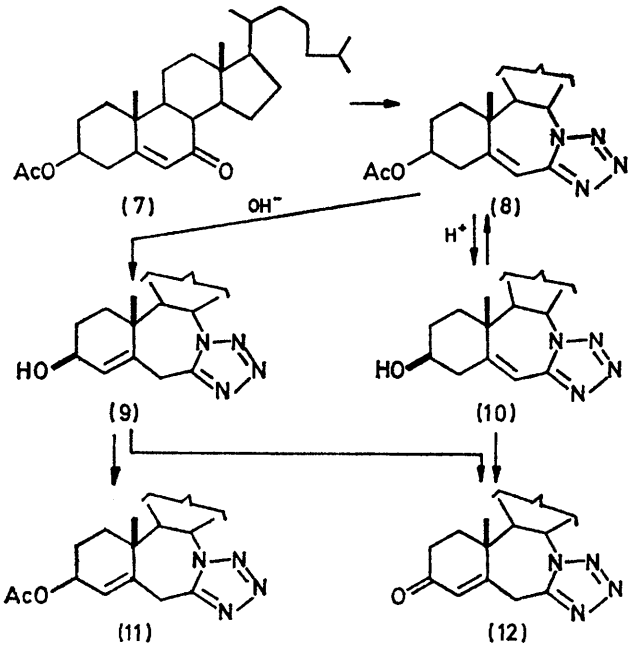
On treatment of 7-oxocholest-5-en-3β-yl acetate (7) with hydrazoic acid–boron trifluoride in chloroform, a tetrazole was obtained in 58% yield. It exhibited a u.v. maximum at 241 nm, and the i.r. spectrum, in addition to ester C=O stretching at 1737 cm^{-1} and C–O stretching at 1242, showed bands at 1665, 1505, 1465, and 1370 cm^{-1}. In the n.m.r. spectrum there were two broad multiplets at δ 4·25 (1H) and 4·75 (1H), which may be ascribed to 8β- and 3α-protons, respectively. The vinylic proton signal at δ 6·62 was downfield as compared with the corresponding signal at δ 5·78 in the αβ-unsaturated ketone (7). These data in the light of the foregoing discussions favour assignment of 7a-aza-B-homo-5-eno[7a,7-*d*]tetrazole structure (8) to the product.

When hydrolysis of (8) was carried out with methanolic potassium hydroxide there resulted a product which lacked the u.v. maximum for the chromophore in (8). In the n.m.r. spectrum the multiplet for 3α-H was shifted upfield to δ 4·02, and the vinylic proton at δ 5·68 appeared as a doublet (*J* 2·5 Hz), indicating that the double bond was between positions 4 and 5. That an allylic shift had occurred and that the hydrolytic product was (9), was also apparent from a two-proton broad singlet at δ 3·72 corresponding to the 6-protons.

The appearance of the C-18 methyl signal as a doublet at δ 0·82 in (9) and (11), with *J* values 2 and 1·3 Hz,

[8] L. A. Lee and J. W. Wheeler, *J. Org. Chem.*, 1972, **37**, 349.
[9] J. N. Shoolery and M. T. Rogers, *J. Amer. Chem. Soc.*, 1958, **80**, 5121.
[10] J. Moural and K. Syhora, *Coll. Czech. Chem. Comm.*, 1970, **35**, 2018.
[11] C. S. Barnes, D. H. R. Barton, J. S. Fawcett, and B. R. Thomas, *J. Chem. Soc.*, 1952, 2339.

respectively, was interesting: possibly the spatial arrangement of rings A and B in these compounds affects the rest of the molecule, and places the C-18



methyl group in a disposition favouring W long range coupling with 17α-H.

Acidic hydrolysis of (8), however, proceeded without double bond shift, and the product showed a u.v. maximum at 245 nm, and the n.m.r. signals for 3α-H [δ 3·75(m)], 8β-H [δ 4·25 (m)], and 6-H [δ 6·63br (s)], supported the structure (10). Acetylation gave back the ester (8).

Both the hydrolysis products (9) and (10) yielded on Oppenauer oxidation the same αβ-unsaturated ketone (12).

EXPERIMENTAL

Optical rotations were measured for solutions in chloroform. U.v. and i.r. spectra were obtained for solutions in ethanol and for potassium bromide discs, respectively. N.m.r. spectra (60 MHz) were recorded in deuteriochloroform containing tetramethylsilane as internal reference. T.l.c. was carried out on silica gel G (Merck) and plates were developed by exposure to iodine vapour. Anhydrous sodium sulphate was employed as drying agent.

17β-*Acetamido*-3-*aza*-A-*homoandrost*-4a-*eno*[3,4-d]*tetrazole* (2).—To a solution (225 ml) of hydrazoic acid in chloroform at 0°, prepared as before,[2] boron trifluoride–ether complex (2·3 ml) was added with shaking. A solution of progesterone (1) (3 g) in chloroform (60 ml) was then added during 4 h at 0°. The mixture was left at room temperature (25—30°) for 20 h and then worked up in the usual way. The residue (3·3 g) obtained gave five spots on t.l.c. (chloroform–methanol, 9 : 1). A solution of the residue in acetone was treated with ether to give a white precipitate (0·9 g), m.p. 290—293°, which on repeated crystallisation from acetone gave a pure *product* (2) (0·4 g, 11%), m.p. 312—314° (decomp.), $[\alpha]_D^{25}$ −9·2° (*c* 0·54), $\lambda_{max.}$ 243 nm (log ε 4·22), $\nu_{max.}$ 3335, 2940, 1670, 1650, 1530, 1450, and 1375 cm^{-1}, δ 0·75 (3H, s), 1·24 (3H, s), 1·97 (3H, s), 3·92 (1H, m), 4·48 (2H, m), 5·45 (1H, m) and 6·50 (1H, s), M^+, 369 (Found: C, 68·15; H, 8·2; N, 18·95. $C_{21}H_{31}N_5O$ requires C, 68·25; H, 8·45; N, 18·95%).

17β-(5-*Methyltetrazol*-1-*yl*)-3-*aza*-A-*homoandrost*-4a-*eno*-[3,4-d]*tetrazole* (4).—After separation of the product (2) all the mother liquors were combined and the residue (2·3 g) thus obtained was chromatographed on a column of alumina (200 g) in chloroform–benzene (1 : 3). Elution with chloroform–benzene (1 : 3) yielded a residue (0·4 g), m.p. 264—267°, which on t.l.c. (chloroform–methanol, 9 : 1) gave two spots. The residue was crystallised from acetone to give a pure *product* (4) (0·13 g, 3%), m.p. 275—278°, $[\alpha]_D^{25}$ +25·27° (*c* 0·12), $\lambda_{max.}$ 243 nm (log ε 4·23), $\nu_{max.}$ 2935, 1650, 1520, 1450, 1400, and 1390 cm^{-1}, δ 0·80 (3H, s), 1·27 (3H, s), 2·54 (3H, s), 4·15 (1H, m), 4·50 (2H, m), and 6·51 (1H, s), M^+, 394 (Found: C, 64·05; H, 7·5; N, 28·3. $C_{21}H_{30}N_8$ requires C, 63·95; H, 7·65; N, 28·4%).

No other pure products could be obtained from the subsequent elutions or mother liquors by crystallisation or preparative t.l.c.

The Tetrazole (2) *from* 17β-*Acetamidoandrost*-4-*en*-3-*one* (3).—17β-Acetamidoandrost-4-en-3-one (3) (0·5 g) prepared by the published procedure [6] was treated with hydrazoic acid solution [2] (20 ml) and boron trifluoride–ether complex (0·3 ml) in the usual way. The crude residue on crystallisation gave the pure product (2) (0·2 g, 36%) m.p. 312—314° (decomp.), with spectra identical with those of the sample obtained above.

7a-*Aza*-B-*homocholest*-5-*eno*[7a,7-d]*tetrazol*-3β-*yl Acetate* (8).—To a hydrazoic acid solution (150 ml) in chloroform at 0°, boron trifluoride–ether complex (5 ml) was added with shaking. A slight turbidity appeared. A solution of 7-oxocholest-5-en-3β-yl acetate (7) (5 g) in chloroform (100 ml) was added to hydrazoic acid solution during 4 h at 0°. The mixture was kept at 25—30° for 40 h and processed in the usual way to obtain a brown residue (4·5 g). The residue was chromatographed over an alumina (100 g) column in benzene. Elution with benzene gave a solid (3·5 g, 58%), m.p. 148—150°, which on crystallisation from methanol afforded the *product* (8), m.p. 154—155°, $[\alpha]_D^{25}$ −137·1° (*c* 0·62), $\lambda_{max.}$ 241 nm (log ε 4·00), $\nu_{max.}$ 2925, 1737, 1665, 1505, 1465, 1370, and 1242 cm^{-1}, δ 0·84 (3H, s), 1·27 (3H, s), 2·05 (3H, s), 4·25 (1H, m), 4·75 (1H, m), and 6·62 (1H, s) (Found: C, 72·5; H, 9·55; N, 11·6. $C_{29}H_{46}N_4O_2$ requires C, 72·15; H, 9·6; N, 11·6%).

Alkaline Hydrolysis of the Acetate (8).—A solution of (8) (2 g) in methanol (30 ml) containing potassium hydroxide (0·6 g) was refluxed for 1 h. The mixture was acidified with glacial acetic acid, concentrated to 5 ml, and poured into ice-cold water. The precipitate was collected, dried, and crystallised from methanol to yield 7a-*aza*-B-*homocholest*-4-*eno*[7a,7-d]*tetrazol*-3β-*ol* (9) (1·5 g, 82%), m.p. 235—236°, $[\alpha]_D^{25}$ −89·13° (*c* 0·56), $\nu_{max.}$ 3360, 2942, 1530, 1465, 1389, and 1370 cm^{-1}, δ 0·82 (3H, d, *J* 2 Hz), 1·33 (3H, s), 2·22 (1H, D_2O exchangeable), 3·72br (2H, s), 4·02 (1H, m), 4·35 (1H, m), and 5·68 (1H, d, *J* 2·5 Hz) (Found: C, 73·35; H, 9·7; N, 12·75. $C_{27}H_{44}N_4O$ requires C, 73·6; H, 10·05; N, 12·7%).

A mixture of the tetrazole (9) (0·1 g), pyridine (0·2 ml), and acetic anhydride (0·1 ml) was heated on a steam-bath for 90 min. The mixture was cooled and poured into ice-cold water, and then processed in the usual way to obtain a residue, which on crystallisation from acetone–light petroleum gave 7a-*aza*-B-*homocholest*-4-*eno*[7a,7-d]*tetrazol*-3β-*yl acetate* (11) (0·07 g, 72%), m.p. 173—174°, $\nu_{max.}$ 2945,

1735, 1720, 1530, 1460, 1380, 1370, and 1245 cm^{-1}, δ 0·82 (3H, d, *J* 1·3 Hz), 1·31 (3H, s), 2·03 (3H, s, Ac), 3·72br (2H, s, 6-H_2), 4·38 (1H, m), 5·03 (1H, m, 3α-H), and 5·63 (1H, d, *J* 3·3 Hz, 4-H) (Found: C, 71·95; H, 9·45; N, 11·5. $C_{29}H_{46}N_4O_2$ requires C, 72·15; H, 9·6; N, 11·6%).

Acid Hydrolysis of the Acetate (8).—To a refluxing solution of the tetrazole (8) (0·1 g) in 95% ethanol (10 ml) was added 6N-hydrochloric acid (0·6 ml) and the mixture refluxed further for 8 h. The reaction mixture was then concentrated until slight turbidity appeared and allowed to cool. The crystalline material which separated was collected and washed with a little ice-cold ethanol and crystallised from ethanol to give 7a-*aza*-B-*homocholest*-5-*eno*[7a,7-d]*tetrazol*-3β-*ol* (10) (0·075 g, 82%), m.p. 142—144°, $[\alpha]_D^{25}$ −151·9° (*c* 0·53), $\lambda_{max.}$ 245 nm (log ε 4·06), $\nu_{max.}$ 3310, 2920, 2870, 1663, 1510, 1465, 1380, and 1373 cm^{-1}, δ 0·82 (3H, s), 1·33 (3H, s), 2·12br (1H, s, D_2O exchangeable), 3·75 (1H, m), 4·25 (1H, m), and 6·63br (1H, s) (Found: N, 12·9. $C_{27}H_{44}N_4O$ requires N, 12·7%).

A mixture of the tetrazole (10) (0·5 g), pyridine (1 ml), and acetic anhydride (0·5 ml) was heated on a steam-bath for 90 min and then poured into ice-cold water. The residue obtained from the usual work-up was crystallised from methanol to yield acetate (8) (0·4 g, 82%).

7a-*Aza*-B-*homocholest*-4-*eno*[7a,7-d]*tetrazol*-3-*one* (12).—(*a*) The tetrazole (9) (0·5 g) and cyclohexanone (5 ml) were added to dry toluene (45 ml) from which 5 ml had been distilled off. The distillation was continued and a solution of aluminium isopropoxide (0·5 g) in dry toluene (15 ml) was added dropwise during 30 min. After distilling off toluene (20 ml), the mixture was kept under reflux for 4 h, cooled, filtered, and the residue washed with toluene (10 ml). The combined toluene layer was steam-distilled, and on cooling extracted with ether (5 × 50 ml). The combined ether extract was washed with 10% hydrochloric acid and water successively, dried, and evaporated to leave an oily residue (0·4 g). Crystallisation from methanol gave *ketone* (12) (0·3 g, 60%), m.p. 184—186°, $[\alpha]_D^{25}$ −76·9° (*c* 0·65), $\lambda_{max.}$ 235 nm (log ε 4·14), $\nu_{max.}$ 2930, 2867, 1675, 1625, 1530, 1462, and 1387 cm^{-1}, δ 0·83 (3H, s), 1·17 (3H, s), 4·05 (2H, s, 6-H_2), 4·55 (1H, m, 8β-H), and 5·89 (1H, s, 4-H) (Found: C, 74·3; H, 9·3; N, 13·15. $C_{27}H_{42}N_4O$ requires C, 73·95; H, 9·65; N, 12·75%).

(*b*) The tetrazole (10) (0·5 g) was submitted to Oppenauer oxidation as in (*a*). Crystallisation of the product from methanol yielded tetrazole (12) identical with the sample obtained in (*a*).

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