Photoluminescence Studies of Molecular Interactions. Part I. Synthesis of Some Indolyl Ketones and Tryptophan Analogues

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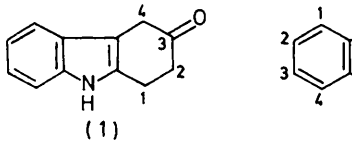
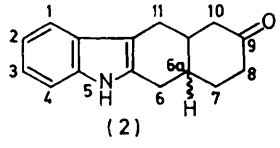
A number of indolyl ketones and an amino-acid have been prepared by Fischer indolisation of cyclic diketones and of their monoethylene acetals: the difficultly accessible 1,2,4,9-tetrahydrocarbazol-3-one (1) and the novel amino-acid 3-amino-1,2,3,4-tetrahydrocarbazole-3-carboxylic acid (5) were obtained in good yield in this way. The direction of indolisation of decalin-2,6-dione monoethylene acetal and of 5α- and 5β-androstane-3,17-diones was established by mass spectrometry and shown to give linearly fused tetrahydrocarbazole derivatives.

PHOTOLUMINESCENCE properties of tryptophan have been used for some time in protein studies,[1-6] and in the analysis of tryptophan derivatives. However, the dependence of these properties on certain structural and physical parameters, although well documented, is only poorly understood. Even less well understood are the photophysical processes occurring in tryptophan and its derivatives. For example, the fluorescence quantum yield for tryptophan [4] in methanol is 0·12 as compared with 0·29 for indole.[5] Since 3-ethyl- and 3-n-heptyl-indoles have quantum yields of 0·33 and 0·36 respectively,[5] the reduced fluorescence efficiency of tryptophan must be associated with the presence of the carboxy- and amino-groups and not merely due to the alkyl substitution. It has been postulated that in polar solvents, the presence of carbonyl groups near the indole nucleus results in the quenching of indole fluorescence.[6]

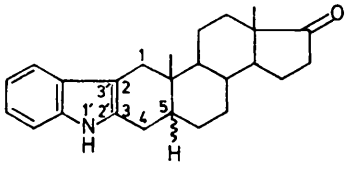
As part of a general study on the photoluminescence of proteins containing tryptophan, a number of indolyl ketones [(1)—(4)] and an amino-acid (5), containing carbonyl groups at various distances from the indole nucleus, were synthesised to serve as test compounds for the above hypothesis. Attempts to prepare the ketone



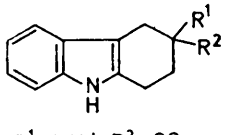
(1)



(2)

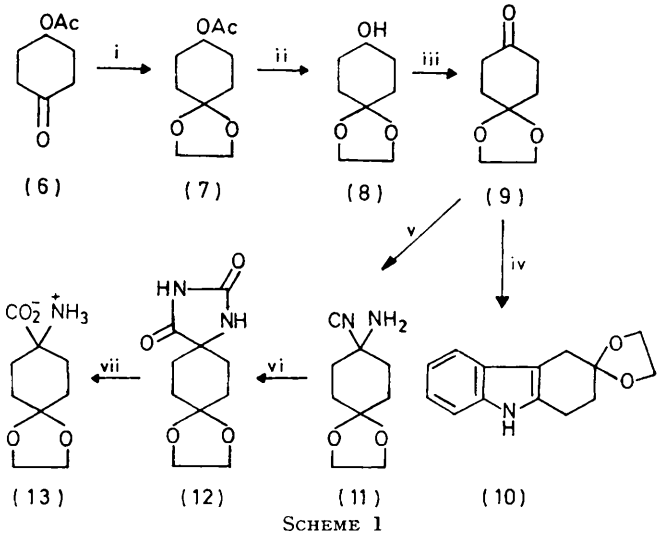


(3) 5-α
(4) 5-β



(5) $R^1 = NH_3^+$, $R^2 = CO_2^-$
(4) $R^1 = NH{\cdot}COCH_3$, $R^2 = CO_2H$

(1) from the bisphenylhydrazone of cyclohexane-1,4-dione,[7] did not give any of the desired product, and this reaction was abandoned since the yield of (1) quoted for this route was only *ca.* 10%. Instead, the ethylene acetal (9) of cyclohexane-1,4-dione was prepared from 4-acetoxycyclohexanone (6) by the route shown in Scheme 1. The formation of the hydroxyacetal (8) directly from 4-hydroxycyclohexanone occurred in lower yields than by the route in Scheme 1. The acetal-ketone (9) was converted into its phenylhydrazone which,



SCHEME 1

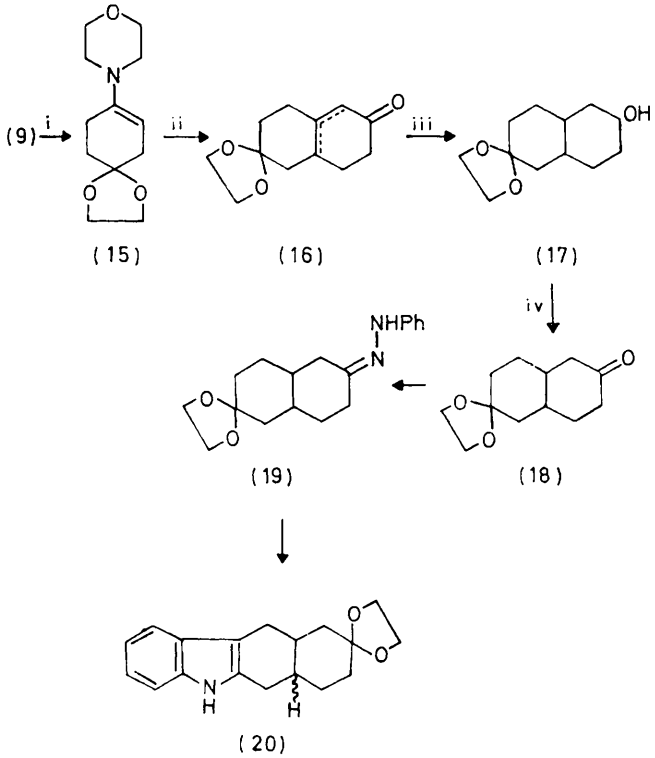
Reagents: i, $(CH_2OH)_2$, TsOH, Δ; ii, ^-OH; iii, CrO_3; iv, $PhNHNH_2$, $ZnCl_2$; v, KCN, NH_3; vi, $(NH_4)_2CO_3$; vii, $Ba(OH)_2$

without characterisation, was used in the Fischer indole synthesis. When subjected to acid catalysed indolisation in aqueous media (H_2O, EtOH, AcOH, or HCO_2H) (9) gave dark tars from which low yields of (10) were obtained after chromatography. However, indolisation in anhydrous benzene with freshly fused $ZnCl_2$ as catalyst gave a 74% yield of the acetal (10), which was hydrolysed quantitatively to the required ketone (1). The u.v. absorption of (1) in neutral, alkaline, and dilute acidic ethanolic solutions was similar to that of 1,2,3,4-tetrahydrocarbazole. The C=O i.r. absorption in Nujol was at 1703 cm^{-1}. Evidently the keto-form is preferred for this product in polar solvents and in the solid state. The acetal-ketone (9), in the Bucherer modification of the Strecker amino-acid synthesis,[8] gave the acid (13) (Scheme 1). Under the acid catalysed conditions of the Fischer indolisation, the acid (13) underwent hydrolysis

[1] R. F. Chen, 'Practical Fluorescence, Theory, Methods and Techniques,' Ed. G. G. Guilbault, Dekker, New York, 1973.
[2] S. V. Konev, 'Fluorescence and Phosphorescence of Proteins and Nucleic Acids,' Plenum Press, New York, 1967.
[3] Pill-Soon Song and W. E. Kurtin, *J. Amer. Chem. Soc.*, 1969, **91**, 4892.
[4] R. F. Chen, *J. Res. Nat. Bur. Stand., Sect. A*, 1973, **76A**, 593.
[5] A. Z. Britten and G. Lockwood, unpublished results.
[6] R. W. Cowgill, *Biochim. Biophys. Acta*, 1970, **200**, 18.
[7] J. Harley-Mason and E. H. Pavri, *J. Chem. Soc.*, 1963, 2504.
[8] J. P. Greenstein and M. Winitz, 'Chemistry of the Amino Acids,' John Wiley, London, 1961.

and indole formation to give the novel tryptophan analogue (5), which was further characterised as its *N*-acetyl derivative (14).

The acetal-ketone (9) was also used to prepare the monoethylene acetal (18) of decalin-2,6-dione (Scheme 2). The alcohol (17) was obtained as a mixture of *cis–trans* isomers. After oxidation to (18), treatment with phenylhydrazine gave an oily product which yielded a small amount of crystalline material which was homogeneous to t.l.c., and had well resolved, sharp i.r. bands and a relatively sharp m.p.: it was concluded that this was one of the isomeric phenylhydrazones (19). Reaction of this in dry benzene with $ZnCl_2$ gave the acetal (20), which was hydrolysed smoothly in acidic aqueous acetone to yield 5,6,6a,7,8,10,10a,11-octahydrobenzo[*b*]-carbazole-9-one (2). The stereochemistry of ring fusion of the carbocyclic residue of compounds (2) and (17)—(20) was not determined. The indolosteroids (3) and (4) were obtained in quantitative yield from a modified Fischer indole reaction on 5α- and 5β-androstane-3,17-dione. It was expected that owing to the greater steric hindrance at position 17, the 3-oxo-group would be more reactive. This was found to be so, since dropwise addition of an ethanolic solution of phenylhydrazine hydrochloride to a boiling ethanolic solution of the steroid produced a

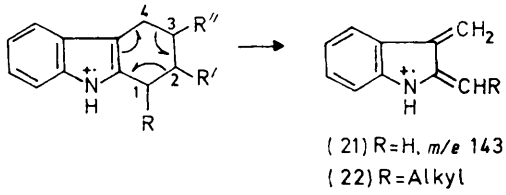


Scheme 2

Reagents: i, morpholine, $-H_2O$; ii, methyl vinyl ketone; iii, Li,NH_3; iv, CrO_3

precipitate within 5 min: in each case this was a single pure product in excellent yield. Absorption at 1730 cm^{-1} in both (3) and (4) confirmed the presence of an intact 17-oxo-group and an intense, sharp band at 3400 cm^{-1} is the characteristic indole absorption. The u.v. absorptions of (3) and (4) were typically representative of the indole chromophore.

That indolisation of the decalin-2,6-dione and of the steroid ketones had proceeded to give linearly fused products, a reduced benzo[*b*]carbazole and androsteno-[3,2-b]indoles respectively, was deduced from mass spectral fragmentation patterns of (1)—(4), (10), and (20). These compounds are all 2,3-disubstituted 1,2,3,4-tetrahydrocarbazoles and as such should fragment by a reverse Diels–Alder pathway (Scheme 3) to give an intense, or even a base peak at *m/e* 143 (21): this has been found [9] for 1,2,3,4-tetrahydrocarbazole itself. This



Scheme 3

fragment is the base peak for compounds (2)—(4) and (10) and occurs at 89 and 24% relative abundance for (20) and (1), respectively. If the products were fused at 1,2-positions then the fragmentation product (22) would be substituted by an alkyl residue and its *m/e* value would be correspondingly increased. The expected [10] loss of keten, CH_2=C=O, from (1), by a reverse Diels–Alder mechanism, leading to the ion *m/e* 143, was apparently suppressed by the preferred formation of fragments of *m/e* 156 and 157. High resolution mass determination of these ions showed their molecular compositions to be $C_{11}H_{10}N$ and $C_{11}H_{11}N$, respectively. These ions correspond to the loss of (CO + H) and CO respectively.

In conclusion, it was found from preliminary experiments that the fluorescence efficiency of the indole nucleus in the steroidal products (3) and (4) was close to that of indole itself. The presence of the carbonyl group in these compounds apparently had no quenching effect. In the structures (1), (2), and (5), the indole fluorescence was quenched to a different extent in each case. However, in none of these compounds was the fluorescence quenched to the same degree as found for 4-(indol-3-yl)butan-2-one, which was practically non-fluorescent. The nature of the quenching mechanism of these indolyl ketones is being examined and the results will be reported later.

EXPERIMENTAL

M.p.s were determined on an Electrothermal apparatus. I.r. spectra were recorded with a Unicam SP 200 spectrophotometer and u.v. spectra were obtained with Beckman Acta V and Unicam SP 8000 instruments. The mass spectra were obtained with an A.E.I. MS9 spectrometer

[9] 'Atlas of Mass Spectral Data,' ed. E. Stenhagen, Interscience, London, 1969, p. 1141.
[10] J. H. Bowie, *Austral. J. Chem.*, 1966, **19**, 1619.

employing direct sample insertion into the ion source (temp. 200—250°) and using an ionising potential of 70 eV.

4,4-*Ethylenedioxycyclohexyl Acetate* (7).—4-Acetoxycyclohexanone (50 g),[11] ethanediol (60 ml), and toluene-*p*-sulphonic acid (1·0 g) were refluxed in dry benzene (400 ml) with vigorous stirring, under water separation. After 8 h, 9—10 ml water had collected (some ethanediol also came over), and the benzene was distilled off. The residual oil was taken up in chloroform, washed twice with water, and dried (K_2CO_3). The chloroform was removed under reduced pressure, yielding *acetal* (7) (51·3 g, 80%). An analytical sample was prepared by distillation, b.p. 94—96° at 1—2 mmHg, $n_D^{23·5}$ 1·4642 (Found: C, 60·1; H, 8·1. $C_{10}H_{16}O_4$ requires C, 60·0; H, 8·05%), *m/e* 43 (71%), 86 (42), 87 (28), 91 (33), 99 (100), 140 (51), and 155 (15).

4,4-*Ethylenedioxycyclohexanol* (8).—The acetal (7) (48·2 g) was refluxed with KOH (24 g) in methanol (250 ml) containing water (30 ml) for 4 h. About ⅔ of the methanol was then removed by distillation, the liquid was cooled, and water (250 ml) was added. The solution was extracted with chloroform (5 × 50 ml), and the extracts were dried ($MgSO_4$) and evaporated under reduced pressure, yielding the hydroxy-acetal (8) (37·7 g) as an oil (75% after distillation), $\nu_{max.}$ (liq. film) 760, 930, 1035, 1105, and 3450 cm^{-1}. On treatment with *p*-nitrobenzoyl chloride in the usual manner, this alcohol formed its p-*nitrobenzoate* as white crystals, m.p. 102·5—103·5° (Found: C, 58·5; H, 5·4; N, 4·55. $C_{15}H_{17}NO_6$ requires C, 58·65; H, 5·6; N, 4·55%).

4,4-*Ethylenedioxycyclohexanone* (9).—Chromium trioxide (24 g; AnalaR), vacuum dried at 80° overnight, was added with stirring to methylene dichloride (600 ml; P_2O_5 dried) containing pyridine (37·96 g; NaOH dried). The dark liquid was cooled to 0° whilst stirring for 20 min. The hydroxy-acetal (8) (6·32 g) was added in one portion, and the liquid was stirred a further 20 min at 0°, and then allowed to warm to room temperature. The dark liquid was decanted from the tarry residue, which was washed four times with ether. The combined organic solutions were washed with saturated aqueous NaCl containing 5% NaOH (4×), neutral saturated NaCl (4×), then dried (K_2CO_3). Evaporation under reduced pressure yielded an oil which crystallised on cooling. This was taken up in ether and the black solid impurities were filtered off to give a pale yellow solution. Reduction of volume and addition of light petroleum (b.p. 60—80°) to turbidity yielded, on standing, white crystals of the *product* (9) (5·14 g, 82%). Scaling up of this reaction gave lower yields. M.p. 71—73° (Found: C, 61·8; H, 7·85. $C_8H_{12}O_3$ requires C, 61·5; H, 7·75%).

4,4-*Ethylenedioxycyclohexanone Phenylhydrazone*.—The acetal (9) (6·0 g) and phenylhydrazine (4·3 g) were separately dissolved in minimal volumes of water, and then mixed. The resulting milky emulsion was extracted with ethyl acetate (5×), and the extract was dried ($MgSO_4$) and evaporated under reduced pressure to give a quantitative yield of the hydrazone which was not further purified.

3,3-*Ethylenedioxy*-1,2,3,4-*tetrahydrocarbazole* (10).—The foregoing phenylhydrazone (9·4 g) was dissolved in dry benzene (250 ml) to which freshly fused and powdered zinc chloride (*ca.* 5 g) was added. The mixture was refluxed 90 min under a Dean–Stark apparatus. Most of the benzene was distilled off; an excess of 10% NaOH was added, and the liquid was extracted with ethyl acetate. The extract was washed with 5% NaOH and then water, dried ($MgSO_4$), and evaporated, yielding a dark solid. Trituration with ether gave the product as a buff coloured solid. The mother liquors on passing through a column of neutral alumina, eluting with ether, yielded a further amount of white solid *product* (10), total yield 6·43 g (74%), m.p. 145·5—147° (from ether–petroleum), *m/e* 143 (100%), 144 (13), and 229 (22) (Found: C, 73·1; H, 6·65; N, 5·95. $C_{14}H_{15}NO_2$ requires C, 73·35; H, 6·6; N, 6·1%).

1,2,4,9-*Tetrahydrocarbazol*-3-*one* (1).—The above ethylene acetal (10) (6·03 g) and toluene-*p*-sulphonic acid (1·4 g) were refluxed in acetone (150 ml) 4 h. The acetone was distilled off, and the residue was taken up in ethyl acetate, washed thrice with $NaHCO_3$ solution, once with water, dried ($MgSO_4$), and taken to low volume. Addition of ether and light petroleum (b.p. 60—80°) yielded, on scratching, a buff solid (5·2 g). This was purified by suspending in dilute HCl containing a little methanol to facilitate wetting, warming to reflux then cooling, extracting with ethyl acetate, and passing through a column of neutral alumina, eluting with ether–ethyl acetate (1 : 1). To the eluate was added a little light petroleum, and cooling to 0° for 2 h yielded the product (1) as a white solid which was washed with ether and light petroleum, m.p. 158—159° (lit.,[7] 148—150°), *m/e* 130 (20%), 143 (24), 156 (100), 157 (70), 185 (86), and 186 (14).

6,6-*Ethylenedioxydecalin*-2-*one Phenylhydrazone* (19).—The acetal (9) (3·12 g) was dissolved in dry benzene (120 ml), morpholine (3·5 g) was added, and the mixture was refluxed overnight under water separation. After 14 h, *ca.* 0·3 ml water had collected; the benzene was distilled off, leaving the morpholine-enamine (15) as an oil which was not further purified.

The foregoing enamine (15) was stirred in dry benzene (25 ml) and methyl vinyl ketone (1·4 g) in benzene (2 ml) was added dropwise at room temperature. After stirring 30 min, the solution was refluxed (3·5 h). The benzene was removed under reduced pressure, methanol (25 ml) and water (25 ml) were added, and the mixture was refluxed overnight. Most of the methanol was distilled off, water was added, and the mixture thrice extracted with ether. The extracts were washed with acidified NaCl solution, dried ($MgSO_4$), and evaporated, yielding an amber oil, which was a mixture of the octalones (16).

The foregoing octalone acetal mixture in dry ether (100 ml) was added dropwise over 10 min to liquid ammonia (300 ml) containing lithium (2·0 g). The mixture was stirred a further 5 min, then ammonium chloride (20 g) was added in portions. The ammonia was allowed to evaporate, water (200 ml) was added, and the solution was thrice extracted with ether. The extracts were dried ($MgSO_4$) and evaporated, yielding as a yellow viscous oil, 6,6-*ethylenedioxydecalin*-2-*ol* (17), *m/e* 55 (10%), 86 (28), 99 (100), 125 (45), 126 (11), and 210 (12).

The hydroxy-acetal (17) was oxidised as described for compound (9), using CrO_3 (12 g), pyridine (19·0 g), and CH_2Cl_2 (300 ml). The product was passed down a column of basic alumina, eluting with ether, yielding (18) as an oil (1·55 g).

Phenylhydrazine (0·8 g) in a little methanol was added to the acetal (18) (1·55 g), dissolved in methanol containing a little water. The mixture was warmed, water (20 ml) was added, and the methanol was distilled off. A viscous yellow oil separated, which was washed with water. The oil solidified on cooling to a waxy solid. This was taken up in ether. Trituration induced the crystallisation of the

[11] J. B. Aldersley, G. N. Burkhardt, A. E. Gillam, and N. C. Hindley, *J. Chem. Soc.*, 1940, 10.

product (19) (310 mg, 14%), m.p. 115—118°, *m/e* 39 (23%), 41 (32), 55 (27), 65 (32), 77 (38), 86 (32), 92 (31), 93 (82), 99 (100), 125 (26), 300 (85), and 301 (20) (Found: M^+, 300·1838. $C_{18}H_{21}N_2O_2$ requires M, 300·1847).

9,9-*Ethylenedioxy*-6,6a,7,8,9,10,10a,11-*octahydro*-5H-*benzo*[b]*carbazole* (20).—Freshly fused, finely powdered zinc chloride (*ca.* 0·5 g) was added to the foregoing phenylhydrazone (19) (310 mg) dissolved in dry benzene (30 ml). After refluxing 90 min, the mixture was left at room temperature 90 min. The benzene was then removed under reduced pressure, 10% NaOH solution (15 ml) was added, and the mixture extracted with ethyl acetate. The organic layer was dried ($MgSO_4$) and evaporated, yielding a buff solid which was washed with ether, giving the *product* (20) (100 mg), m.p. 191—193°, *m/e* 32 (33%), 41 (22), 43 (36), 44 (25), 55 (21), 86 (27), 99 (100), 125 (46), 140 (31), 143 (89), 154 (36), 168 (34), 182 (26), and 283 (83) (Found: M^+, 283·1572. $C_{18}H_{21}NO_2$ requires M, 283·1578).

5,6,6a,7,8,10,10a,11-*octahydrobenzo*[b]*carbazol*-9-*one* (2).—A solution of the acetal (20) (100 mg) in acetone (10 ml) containing 2M-HCl (1 ml) was refluxed 50 min. The acetone was distilled off, until the volume reached *ca.* 5 ml, when a white crystalline precipitate formed. The mixture was cooled to 0°, and the white solid filtered off, washed with water, and dried *in vacuo*, to give the *product* (2), m.p. 234—236°, *m/e* 130 (11%), 143 (100), 144 (15), 168 (11), 239 (71), 240 (13) (Found: M^+, 239·1310. $C_{16}H_{17}NO$ requires M, 239·1311).

1-*Amino*-4,4-*ethylenedioxycyclohexanecarbonitrile* (11).—The acetal (9) (3·0 g), NH_4Cl (3·0 g), KCN (3·0 g), and ammonia solution (*d* 0·880; 4·5 ml) were stirred (4 days) in water (25 ml) containing methanol (3 ml). Water (25 ml) was then added and the solution was extracted with chloroform 4 times. The extract was dried ($MgSO_4$) and evaporated, the residue being recrystallised from ether–light petroleum (b.p. 60—80°) to give the product (11) (2·45 g), with a second crop (0·7 g) (total yield 87%), m.p. 39—50° (mixture of isomers) (Found: C, 59·3; H, 7·85; N, 15·2. Calc. for $C_9H_{14}N_2O_2$: C, 59·3; H, 7·75; N, 15·35%).

4,4-*Ethylenedioxycyclohexanespiro*-4′-*imidazolidine*-2′,5′-*dione* (12).—The foregoing aminonitrile (11) (3·0 g) was stirred 3 days with ammonium carbonate (10 g) in water (15 ml) at room temperature. The white solid produced was filtered off, washed with water, and dried at 100° *in vacuo*, giving the *product* (12) (3·37 g, 90·5%), m.p. 245—247° (Found: C, 53·2; H, 6·26; N, 12·35. $C_{10}H_{14}N_2O_4$ requires C, 53·1; H, 6·25; N, 12·4%).

1-*Amino*-4,4-*ethylenedioxycyclohexanecarboxylic Acid* (13).—The hydantoin (12) (4·0 g) and barium hydroxide octahydrate (15·4 g) were heated at 150° in water (150 ml) in a bomb for 6 h. After cooling to room temperature, an excess of solid ammonium carbonate was added, and the mixture was heated on a water-bath for 30 min. This mixture was left at room temperature overnight, and the $BaCO_3$ produced was then filtered off. The filtrate was taken to dryness *in vacuo*, and the residue was crystallised from aqueous methanol, yielding fine needles of the *product* (13), m.p. 301—304° (decomp.) (2·83 g, 80%). This product was shown to be free of Ba^{2+} and NH_4^+ by usual tests (Found: C, 53·45; H, 7·45; N, 7·05. $C_9H_{15}NO_4$ requires C, 55·7; H, 7·5; N, 6·95%).

3-*Amino*-1,2,3,4-*tetrahydrocarbazole*-3-*carboxylic Acid* (5).—To a solution of compound (13) (2·02 g) in water (30 ml) and concentrated HCl (2 ml) was added phenylhydrazine hydrochloride (1·46 g), and the mixture was heated to 100° for 50 min, then refluxed 5 min. After cooling to room temperature and neutralisation with 0·880 ammonia–water (3 : 1) to pH 7·2, copious precipitation occurred. The white plates were filtered off, washed with water and then acetone, and dried 100° *in vacuo*, to give the *product* (5) (1·77 g, 77%), decomp. *ca.* 300° (Found: C, 67·9; H, 6·15; N, 11·95. $C_{13}H_{14}N_2O_2$ requires C, 67·8; H, 6·15; N, 12·15%).

3-*Acetamido*-1,2,3,4-*tetrahydrocarbazole*-3-*carboxylic Acid* (14).—To the amino-acid (5) (1·00 g) dissolved in 2M-NaOH (20 ml) was added acetic anhydride (5 ml) at room temperature in 8 portions, alternately with sufficient NaOH to keep the solution alkaline, with shaking between additions. A white precipitate formed, apparently insoluble in NaOH solution. After cooling in ice–water for 40 min, the solid was filtered off and washed twice with a little water. The solid sodium salt was dissolved in water, a few drops of dilute HCl were added to precipitate the free acid, and this was filtered off and dried, to give solid *product* (14) (0·68 g), m.p. 278—281° (decomp.). The mother liquors deposited further crystals on standing, total yield 90% (Found: C, 66·1; H, 5·8; N, 10·0. $C_{15}H_{16}N_2O_3$ requires C, 66·15; H, 5·9; N, 10·3%).

5α- *and* 5β-*Androst*-2-*eno*[3,2-b]*indol*-17-*one* (3) *and* (4).—5α- or 5β-Androstane-3,17-dione (1·44 g) was heated to reflux in ethanol (45 ml). To the boiling solution was added phenylhydrazine hydrochloride (0·73 g) in water (15 ml), dropwise with stirring. After *ca.* 5 min, the solution became cloudy, and a solid precipitated. The mixture was allowed to cool whilst stirring for 10 min. The white solid product was filtered off, washed with water, and dried *in vacuo*. The yields in each case were almost quantitative. 5α-Product, m.p. 286—289° (decomp.), *m/e* 32 (24%), 143 (100), 144 (19), 361 (45), and 362 (12), 5β-*product*, decomp. 285° without melting (Found: C, 83·3; H, 8·5; N, 4·1. $C_{25}H_{31}NO$ requires C, 83·05; H, 8·65; N, 3·85%), *m/e* 31 (56%), 32 (31), 45 (42), 130 (30), 143 (100), 144 (20), 182 (15), 361 (67), and 362 (19).

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