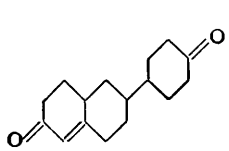
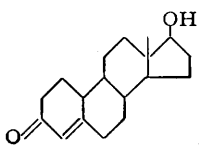
932. *Experiments on the Synthesis of Potential Cortical Hormone Substitutes. Part II.**

By F. J. Dyer, D. A. A. Kidd, and James Walker.

Analogues of deoxycorticosterone (III) have been sought in which the αβ-unsaturated ketone group and α-ketol side-chain characteristic of (III) have been incorporated. These were obtained by applying the Friedel–Crafts reaction to 3-methyl-5-phenyl-*cyclo*hex-2-en-1-one and by suitable alkylation of Hagemann's ester, followed in both cases by appropriate steps. A substance (X; R = Me) was obtained having about 20—30% of the activity of (III) in prolonging the life of bilaterally adrenalectomised rats subjected to cold stress.

Although numerous synthetic œstrogens are now known, only sporadic attempts have been made to prepare synthetic analogues with the undoubtedly more specific biological activities typical of other steroid hormones, such as testosterone, progesterone, or the adrenal cortical hormones. Androgenic activity has, however, been reported for the diketone (I) (Wilds, Shunk, and Hoffman, *J. Amer. Chem. Soc.*, 1949, **71**, 3266) and, still more markedly, for 19-nortestosterone (II) † (Birch, *J.*, 1950, 367; *Ann. Reports*, 1950, **47**, 210), while, at the outset of the work described below, slight activity characteristic of deoxycorticosterone (III) had been observed in two synthetic α-ketols (Linnell and Roushdi, *Quart. J. Pharm. Pharmacol.*, 1941, **14**, 270); in the interval, marked activity typical of (III) has been claimed for further synthetic α-ketols, notably (IV; R = OMe> R = H) and (V) (Linnell, Mathieson, and Williams, *Nature*, 1951, **167**, 237).


(I)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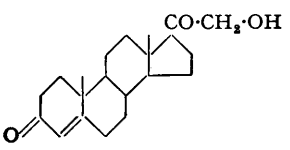
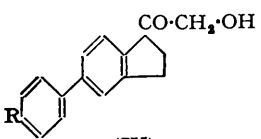
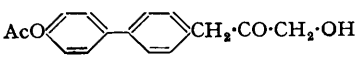
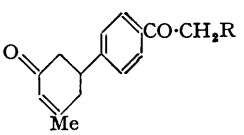

(III)


(IV)


(V)

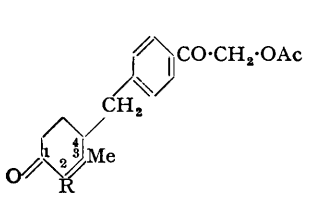

(VI; R = H) (VIII; R = OAc)
(VII; R = Cl) (IX; R = OH)

The structural features, besides the pregnene skeleton, common to the six biologically active adrenal cortical steroids are the αβ-unsaturated ketonic grouping in ring A and the α-ketol group comprising the β-orientated side-chain at position 17 in ring D. We have therefore undertaken the synthesis of analogues containing the latter features separated by approximately the same distance as in (III). The diketone (VI) was obtained from 3-methyl-5-phenyl*cyclo*hex-2-enone, accessible from benzaldehyde and ethyl acetoacetate, by the Friedel–Crafts reaction with acetyl chloride, and the expected orientation was confirmed by oxidation with potassium permanganate to *p*-acetylbenzoic acid. Chloroacetyl chloride similarly afforded the chloro-diketone (VII), which was converted successively into the acetoxy- (VIII) and the hydroxy-diketone (IX). The Sommelet reaction (*Compt. rend.*, 1913, **157**, 852, 1443) was found suitable for the preparation of several cyano- and carboxy-aldehydes intended for use in the Knoevenagel synthesis of substituted 5-aryl*cyclo*hexenones, but either condensation of these aldehydes with ethyl acetoacetate or sub-

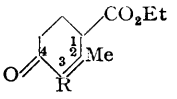
* Part I, *J.*, 1942, 347. † The $C_{(19)}$ angular methyl group is likewise not essential for the full biological activity of progesterone (Miramontes, Rosenkranz, and Djerassi, *J. Amer. Chem. Soc.*, 1951, **73**, 3540).

sequent stages did not proceed as satisfactorily as with benzaldehyde and these alternative routes to compounds of type (IX) were not pursued.

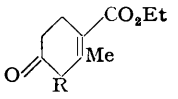
As a closer stereochemical approximation to (III), access was sought to a substance of the type (X), for which the Hagemann ester (XI; R = H) appeared to be a suitable starting material. The structure of Hagemann's ester has been the subject of controversy and still cannot be said to be settled. Although Rabe and Rahm (*Ber.*, 1905, **38**, 969) noted that it contained a potential glutaconic ester system, $-CO\cdot\overset{|}{C}H\cdot\overset{|}{C}:\overset{|}{C}\cdot CO-$, clear evidence of structural isomerism in a compound of this type was first produced for the analogous solid 4-keto-2 : 6-diphenyl*cyclo*hexenecarboxylic ester by Dieckmann (*Ber.*, 1911, **44**, 975; 1912, **45**, 2689), and, by inference, for the liquid Hagemann ester. Dieckmann, however, preferred formula (XI*a*; R = H) on general grounds for the stable ketonic form of the ester, but it should be noted that (XI*b*; R = H) is obviously the form which would be favoured by hyperconjugation and, indeed, is in line with the fact that the first stage in the alkylation of Hagemann's ester affords 3-alkyl derivatives (XI; R = alkyl) (Dieckmann, *Ber.*, 1912, **45**, 2697; Kötz *et al.*, *Annalen*, 1913, **400**, 72), an observation which has received repeated practical application (cf. Cornubert and Maurel, *Bull. Soc. chim.*, 1931, **49**, 1515; Bergmann and Weizmann, *J. Org. Chem.*, 1939, **4**, 266; Schwenk and Bloch, *J. Amer. Chem. Soc.*, 1942, **64**, 3050; Smith and Rouault, *ibid.*, 1943, **65**, 631; Hogg, *ibid.*, 1948, **70**, 161;



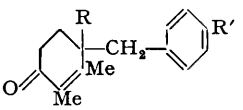
(X)



(XI*a*)



(XI*b*)



(XII; R = CO_2Et, R′ = CN)
(XIII; R = R′ = CO_2H)
(XIV; R = R′ = CO_2Et)
(XV; R = CO_2H, R′ = CO_2Et)
(XVI; R = H, R′ = CO_2Et)

Horning, Horning, and Platt, *ibid.*, 1949, **71**, 1771; Stork and Burgstahler, *ibid.*, 1951, **73**, 3544). The methylation product of Hagemann's ester is therefore (XI; R = Me), which is analogous to an $\alpha\gamma$-dialkylglutaconic ester, and it could undergo further alkylation either at $C_{(1)}$ or at $C_{(3)}$, although, as far as we are aware, a second-stage alkylation of Hagemann's ester has not previously been attempted. Inspection of (XI; R = Me), however, suggests that hyperconjugation would now favour (XI*a*; R = Me) as the more stable form and that the second stage of alkylation would take place at the 1-position. With these considerations in view, Hagemann's ester was first methylated to give (XI; R = Me) and then alkylated with *p*-cyanobenzyl bromide to give ethyl 1-*p*-cyanobenzyl-4-keto-2 : 3-dimethyl*cyclo*hex-2-ene-1-carboxylate (XII). Exhaustive hydrolysis yielded a dibasic acid (XIII) and esterification gave a mixture of neutral (XIV) and half-esters (XV). The colour and ultra-violet light absorption of the 2 : 4-dinitrophenylhydrazone of (XIV) were in accordance with expectation for those of such a derivative of an $\alpha\beta$-unsaturated carbonyl compound, substituted in both the α- and the β-position, although no measurement appears to have been recorded for a ketone of that nature (cf. Braude and Jones, *J.*, 1945, 498). The half-ester (XV) was decarboxylated in quinoline in the presence of copper bronze to give the ester (XVI), and this was converted in the usual manner, *via* the free acid, the acid chloride, and the diazo-ketone into 4-*p*-acetoxyacetylbenzyl-2 : 3-dimethyl*cyclo*hex-2-enone (X; R = Me).

The efficiencies of (VI), (VIII), (IX), and (X; R = Me), in comparison with deoxycorticosterone (III), in protecting bilaterally adrenalectomised rats from cold stress (cf. Selye and Schenker, *Proc. Soc. Exp. Biol. Med.*, 1938, **39**, 518) are shown in the Table. Only (X; R = Me) was definitely found to be active and it had possibly 20—30% of the activity of (III). With the small groups of animals used, however, this assay can only be regarded as a sorting test. None of the compounds now described caused regression of the thymus gland in infantile rats (cf. Bruce, Parkes, and Perry, *Lancet*, 1952, I, 790) according to tests kindly carried out by Mr. John Lock of the Crookes Laboratories.

Cold stress tests on adrenalectomised immature rats.

Compound (in arachis oil)	Total dose per 50 g. rat (given in 2 portions subcutaneously)	Observations	Potency indications (if III = 100) $P = 0{\cdot}95,\ 100 \pm 80$
(III)	0·25 mg.	1/8 dead in 6 hrs. 1/8 ,, 10 ,,	100
(VI)	2·5 mg.	4/8 dead in 6 hrs. 6/8 ,, 10 ,,	$\ll 20$
(VIII)	2·5 mg.	2/8 dead in 6 hrs. 5/8 ,, 10 ,,	< 20
(IX)	2·5 mg.	5/8 dead in 6 hrs.	$\ll 20$
(X; R = Me)	2·5 mg.	0/8 dead in 6 hrs. 0/8 ,, 10 ,,	$\cdot\sim .20$
Controls (receiving arachis oil only)	—	3/7 dead in 6 hrs. 7/7 ,, 10 ,,	—

Experimental

5-p-*Acetylphenyl*-3-*methyl*cyclo*hex*-2-*enone* (VI).—Powdered aluminium chloride (33·8 g., 2 mols.) was added at room temperature during 1 hour to a solution of 3-methyl-5-phenyl-*cyclo*hex-2-enone (21·1 g., 1 mol.) (Knoevenagel, *Annalen*, 1895, **288**, 352) and acetyl chloride (18·6 c.c., 2 mols.) in carbon disulphide (250 c.c.), stirred under reflux and protected from atmospheric moisture. After 2 hours' stirring, the mixture was boiled for 15 minutes and the solvent was then distilled off. The residue was decomposed with crushed ice containing concentrated hydrochloric acid (40 c.c.), and the precipitated product was recovered in ethyl acetate. Fractionation of the extract afforded a viscous syrup, b. p. 176—188°/0·4 mm., which gave a crystalline solid (38%) on treatment with light petroleum. Recrystallisation from aqueous ethanol gave colourless needles of 5-p-*acetylphenyl*-3-*methyl*cyclo*hex*-2-*enone*, m. p. 90—91° (Found: C, 78·5; H, 7·0. $C_{15}H_{16}O_2$ requires C, 78·9; H, 7·0%).

The substance (3 g.) was treated in acetone (150 c.c.) with potassium permanganate (total, 8·3 g.), in small portions, at room temperature with frequent shaking until a permanent excess could be detected. The mixture was diluted with water (150 c.c.) and clarified with sulphur dioxide. After being rendered strongly acid with concentrated hydrochloric acid and chilled, the solution was filtered and thoroughly extracted with ether. Evaporation of the extract yielded a partly crystalline residue, giving crystalline material on treatment with 40% aqueous ethanol. Recrystallisation from aqueous ethanol gave long prisms of *p*-acetylbenzoic acid, m. p. and mixed m. p. with an authentic specimen (see below), 205—206° (Found: C, 65·7; H, 5·0. Calc. for $C_9H_8O_3$: C, 65·8; H, 4·9%).

Ethyl p-*Cyanobenzoylmalonate*.—A solution of *p*-cyanobenzoyl chloride (2·7 g.) (Gangi and Gisvold, *J. Amer. Pharm. Assoc.*, 1949, **38**, 154) in dry benzene was added dropwise to an alcohol-free solution (cf. Bowman, *J.*, 1950, 324) of ethoxymagnesiomalonic ester [from magnesium (0·59 g.), ethanol (2 c.c.), and ethyl malonate (3·9 g.)] in benzene. The mixture was then refluxed for 1½ hours, cooled, and decomposed with ice and dilute sulphuric acid. The residue, left on evaporation of the benzene layer, yielded long, colourless, fine needles of *ethyl* p-*cyanobenzoylmalonate* (2·65 g.), m. p. 63°, on crystallisation from benzene–light petroleum (Found: C, 61·9; H, 5·0; N, 5·0. $C_{15}H_{15}O_5N$ requires C, 62·2; H, 5·2; N, 4·9%).

p-*Acetylbenzoic Acid*.—Ethyl *p*-cyanobenzoylmalonate (5 g.) was heated under reflux with glacial acetic acid (15 c.c.), concentrated hydrochloric acid (5 c.c.), and water (3 c.c.) until evolution of carbon dioxide ceased (*ca.* 12 hours). The solution was evaporated to small bulk *in vacuo*, water was added, and the precipitate was collected and extracted with excess of aqueous sodium hydrogen carbonate. Acidification of the extract and crystallisation from aqueous alcohol gave *p*-acetylbenzoic acid, m. p. 205—206° (lit., in the range 200° to 210°).

5-p-*Chloroacetylphenyl*-3-*methyl*cyclo*hex*-2-*enone* (VII).—A mixture of 3-methyl-5-phenyl-*cyclo*hex-2-enone (9·3 g.) and chloroacetyl chloride (7 g., 1·2 mols.) in carbon disulphide (200 c.c.) was treated with powdered aluminium chloride (14 g., 2·1 mols.) as described above, except that the final period of reflux was 2 hours. The product was recovered in ethyl acetate, as before, and fractionation gave a small fore-running of unchanged 3-methyl-5-phenyl*cyclo*hexenone, b. p. 95—120°/0·5 mm., followed by the main product, b. p. 200—210°/0·4 mm. Crystallisation from aqueous ethanol afforded small colourless prisms of the *chloro-ketone*, m. p. 94—95° (Found: C, 68·2; H, 6·0. $C_{15}H_{15}O_2Cl$ requires C, 68·6; H, 5·7%). Distillation of this compound was accompanied by some decomposition and loss of material. If, however,

the unchanged starting material was first removed by distillation, the residue could be used for the next stage (following experiment) without further purification.

5-p-*Acetoxyacetylphenyl*-3-*methyl*cyclo*hex*-2-*enone* (VIII).—A solution of the foregoing compound (1·1 g.) in glacial acetic acid (25 c.c.) was refluxed with potassium acetate (2 g.) for 6—7 hours. The crude product (1·1 g.) was isolated by removing the solvent *in vacuo* on the water-bath and treating the residue with water. Recrystallisation from alcohol gave colourless needles of the *acetoxyacetyl* compound, m. p. 143—144° (Found: C, 70·8; H, 6·1. $C_{17}H_{18}O_4$ requires C, 71·3; H, 6·3%).

5-p-*Hydroxyacetylphenyl*-3-*methyl*cyclo*hex*-2-*enone* (IX).—Concentrated hydrochloric acid (2 c.c.) was added dropwise to a solution of the above acetate (1 g.) in chloroform (15 c.c.) and methanol (30 c.c.), containing water (3 c.c.), and the mixture was left at room temperature for 40 hours. Addition of water (100—150 c.c.) caused the separation of a chloroform layer which was removed. The aqueous phase was extracted with chloroform and the combined chloroform layers were dried and evaporated. Recrystallisation from a small volume of benzene gave colourless lances (0·42 g.) of the *ketol*, m. p. 127—129° (Found: C, 73·8; H, 6·7. $C_{15}H_{16}O_3$ requires C, 73·8; H, 6·6%).

m-*Cyanobenzaldehyde*.—A suspension of hexamine (7·1 g.) and *m*-cyanobenzyl bromide (5 g.) in chloroform (50 c.c.) was refluxed for 3 hours. When cold, the solid (7·5 g.) was collected, dried in a vacuum, and decomposed with 50% aqueous acetic acid under reflux for 2 hours. The solution was cooled, diluted with an equal volume of water, and chilled. The crop of colourless needles was collected, washed with aqueous sodium hydrogen carbonate, and dried, a further small crop being obtained on extraction of the mother-liquors with ether. The *m*-cyanobenzaldehyde (40%) thus prepared had m. p. 77—79°, alone and in admixture with an authentic specimen.

p-*Cyanobenzaldehyde*.—By a similar procedure *p*-cyanobenzyl bromide (5 g.) afforded the aldehyde (51%), m. p. 99°.

iso*Phthalaldehydic Acid*.—(*a*) *m*-Bromomethylbenzoic acid (3 g.) was allowed to react with hexamine (3·9 g.) as just described. The intermediate quaternary salt (4·7 g.) was decomposed, as before, with 50% aqueous acetic acid, yielding *iso*phthalaldehydic acid (48%), m. p. 170—171°.

(*b*) *m*-Bromomethylbenzoic acid (2·8 g.) and 10% aqueous copper nitrate (50 c.c.) were refluxed for 8 hours and then cooled overnight. The crystalline precipitate of *iso*phthalaldehydic acid (80%), collected, washed with water, and dried, had m. p. 172—173°, alone and in admixture with the product from (*a*).

This aldehyde did not react smoothly with ethyl acetoacetate under the usual conditions.

Diethyl 4-m-*Cyanophenyl*-2 : 6-*diketoheptane*-3 : 5-*dicarboxylate*.—A mixture of *m*-cyanobenzaldehyde (1 g.) and ethyl acetoacetate (2 c.c.) was treated with piperidine (0·5 c.c.) in ethanol (0·5 c.c.) and set aside for 12 hours at room temperature. The solid product (77%; m. p. 139—140°) was collected and washed with ethanol. The pure *dione* separated from ethanol in small colourless needles, m. p. 146—147° (Found: C, 64·4; H, 6·2; N, 3·8. $C_{20}H_{23}O_6N$ requires C, 64·3; H, 6·2; N, 3·7%).

Diethyl 4-p-*cyanophenyl*-2 : 6-*diketoheptane*-3 : 5-*dicarboxylate*, m. p. 183—185° (Found: C, 64·0; H, 5·7; N, 4·0%), was similarly obtained (77%).

Hydrolysis of Diethyl 4-m-*Cyanophenyl*-2 : 6-*diketoheptane*-3 : 5-*dicarboxylate*.—(*a*) The dione (2 g.) was heated for 4 hours under reflux with a mixture of acetic acid (15 c.c.) and sulphuric acid (1 c.c.). The dark solution was cooled, poured on ice, and filtered from a trace of amorphous solid. Extraction with ether gave a clear gum which crystallised slowly on trituration with light petroleum. The product (0·25 g.), which was insoluble in aqueous sodium hydrogen carbonate, separated from 95% alcohol in small colourless prisms, m.p. 167—168°, and appeared to be 5-m-*carbamylphenyl*-3-*methyl*cyclo*hex*-2-*enone* (Found: C, 72·9; H, 6·3; N, 6·2. $C_{14}H_{15}O_2N$ requires C, 73·4; H, 6·5; N, 6·2%). A further crop (0·3 g.) was obtained by concentration of the aqueous phase and further extraction with ether.

(*b*) The dione (3 g.) was treated with 20% aqueous potassium hydroxide at the b. p. for 14—15 hours. Subsequent acidification gave a partly crystalline product, from which a non-ketonic, nitrogen-free acid was isolated by reprecipitation from aqueous sodium hydrogen carbonate. The substance separated from water in prisms, m. p. 191—192°, and was shown by elementary analysis to be β-m-*carboxyphenylglutaric acid* (Found: C, 57·2; H, 5·1. $C_{12}H_{12}O_6$ requires C, 57·2; H, 4·8%).

Ethyl 1-p-*Cyanobenzyl*-4-*keto*-2 : 3-*dimethyl*cyclo*hex*-2-*ene*-1-*carboxylate* (XII).—Ethyl 4-keto-2 : 3-dimethyl*cyclo*hex-2-ene-1-carboxylate (62·4 g.) (Horning, Horning, and Platt, *J. Amer.*

Chem. Soc., 1949, **71**, 1771) in ether was slowly added to a suspension of finely powdered sodium ethoxide (from 7·4 g. of sodium) in dry ether (400 c.c.), stirred in hydrogen. After 2 hours' stirring to complete formation of the sodio-derivative, a solution of *p*-cyanobenzyl bromide (62·4 g.) (Case, *J. Amer. Chem. Soc.*, 1925, **47**, 1144) in ether was gradually added, and the mixture was stirred under hydrogen for a further 10 hours. Two layers separated on addition of water. The aqueous phase was extracted with ether. Fractionation of the combined dried ethereal solutions afforded the *ester* (XII) as a viscous yellow oil (44%), b. p. 200—215°/0·1 mm., 175—180° (bath)/0·005 mm. (Found: N, 5·0. $C_{19}H_{21}O_3N$ requires N, 4·5%). The 2 : 4-*dinitrophenylhydrazone* separated from ethanol in tiny orange-red prisms, m. p. 153—156° (Found: N, 13·8. $C_{25}H_{25}O_6N_5$ requires N, 14·2%).

1-p-*Carboxybenzyl*-4-*keto*-2 : 3-*dimethyl*cyclo*hex*-2-*ene*-1-*carboxylic Acid* (XIII).—A suspension of the above ester (43·5 g.) in 25% aqueous potassium hydroxide (450 c.c.) was boiled under reflux for 48 hours, the oil slowly dissolving with evolution of ammonia. The crude dibasic *acid* separated in quantitative yield on acidification with hydrochloric acid. Recrystallisation from aqueous methanol afforded small colourless prisms, m. p. 233—234° (Found: C, 68·0; H, 6·0. $C_{17}H_{18}O_5$ requires C, 67·5; H, 6·0%).

1-p-*Carbethoxybenzyl*-4-*keto*-2 : 3-*dimethyl*cyclo*hex*-2-*ene*-1-*carboxylic Acid* (XV).—A suspension of the above dicarboxylic acid (48 g.) in benzene (320 c.c.), ethanol (20 c.c.), and concentrated sulphuric acid (6 c.c.) was refluxed for 24 hours. The undissolved solid was then collected and treated in the same manner for a further 24 hours with a fresh mixture of the same composition. After removal of the final small insoluble residue the combined filtrates were washed with water and extracted with aqueous sodium hydrogen carbonate. Fractionation of the washed and dried benzene layer gave the diethyl ester (XIV) as a viscous yellow oil (20·1 g.), b. p. 196—203°/0·03 mm.; the 2 : 4-*dinitrophenylhydrazone* separated from methanol–ethyl acetate in crimson plates, m. p. 193°, $\lambda_{max.}$ 390 mμ (log ε 4·53 in $CHCl_3$) (Found: N, 9·9. $C_{27}H_{30}O_8N_4$ requires N, 10·4%). The sodium hydrogen carbonate washings (above) were acidified and extracted with benzene. Evaporation of the dried extract gave a crystalline residue (22 g.; m. p. 105—110°), and recrystallisation from aqueous ethanol afforded cream-coloured prisms (24%) of 1-p-*carbethoxybenzyl*-4-*keto*-2 : 3-*dimethyl*cyclo*hex*-2-*ene*-1-*carboxylic acid* (XV), m. p. 141—142° (Found: C, 69·1; H, 6·8. $C_{19}H_{22}O_5$ requires C, 69·1; H, 6·7%).

4-*p*-*Carbethoxybenzyl*-2 : 3-*dimethyl*cyclo*hex*-2-*en*-1-*one* (XVI).—A solution of the foregoing half-ester (8·6 g.) in redistilled quinoline (30 c.c.) was refluxed with a trace of copper bronze in a slow stream of nitrogen, and the issuing gases were passed through standard barium hydroxide solution. When titration showed that 1 mol. of carbon dioxide had been liberated (7—8 hours), the cooled mixture was distributed between ether and dilute hydrochloric acid. The ethereal layer was washed successively with dilute hydrochloric acid, water, aqueous sodium hydrogen carbonate, and water. Fractionation of the dried ethereal solution afforded 4-*p*-carbethoxybenzyl-2 : 3-dimethyl*cyclo*hex-2-enone as a yellow oil (54%), b. p 150—160°/0·001 mm., but precise analytical figures could not be obtained (Found: C, 73·9; H, 7·6. Calc for $C_{18}H_{22}O_3$: C, 75·5; H, 7·7%). The substance was characterised as the 2 : 4-*dinitrophenylhydrazone*, which separated from methanol–ethyl acetate in brick-red prisms, m. p. 166° (Found: N, 12·3. $C_{24}H_{26}O_6N_4$ requires N, 12·0%).

4-p-*Carboxybenzyl*-2 : 3-*dimethyl*cyclo*hex*-2-*enone*.—The above ester (5 g.) was treated with 20% aqueous potassium hydroxide solution (50 c.c.) under reflux for 3 hours. Ethanol was added to homogeneity, and refluxing was continued for a further 4 hours. The cooled solution was diluted with water and washed with ether. Acidification of the aqueous phase with hydrochloric acid precipitated a gum, which was isolated in ether and repeatedly digested with boiling light petroleum. The decanted petroleum extracts were combined and evaporated, leaving a solid residue, and recrystallisation from ligroin afforded colourless prisms (2·15 g.) of 4-p-*carboxybenzyl*-2 : 3-*dimethyl*cyclo*hex*-2-*enone*, m. p. 119° (Found: C, 73·8; H, 7·1. $C_{16}H_{18}O_3$ requires C, 74·4; H, 7·0%).

4-p-*Acetoxyacetylbenzyl*-2 : 3-*dimethyl*cyclo*hex*-2-*enone* (X; R = Me).—The above acid (1·6 g.) was dissolved in aqueous sodium hydroxide (1·05 equivalents in 40 c.c.) and the filtered solution was lyophilised at 0·005 mm. The tan-coloured sodium salt was finally dried at 105°/0·001 mm. and powdered under dry benzene (10 c.c.). A few drops of dry pyridine were added and the suspension was cooled in ice while oxalyl chloride (1·15 c.c.) was slowly run in with frequent shaking. Effervescence had ceased after 10 minutes at room temperature and the solvent was removed below 20°. Fresh benzene was added and evaporated in the same way and the residue was stirred with benzene and filtered. The solution of the acid chloride thus prepared was added dropwise to dried ethereal diazomethane (from 1·5 g. of methylnitrosourea),

cooled in ice, and the mixture was set aside at room temperature overnight. On removal of the solvents the syrupy residue was treated with glacial acetic acid (10 c.c.) on the water-bath. After 1½ hours, when evolution of nitrogen had entirely ceased, water (20 c.c.) was added and the product was taken up in ether. The ethereal solution was washed thoroughly with aqueous sodium hydrogen carbonate and with water, and then dried and evaporated. Crystallisation of the residue from methanol containing a little water gave faintly yellow prisms (0·65 g.) of the 4-p-*acetoxyacetylbenzyl* compound, m. p. 98—99° (Found: C, 72·5; H, 7·0. $C_{19}H_{22}O_4$ requires C, 72·6; H, 7·1%).

Biological Tests.—Weanling rats of the hooded strain were bilaterally adrenalectomised under ether and left to recover for a few hours. They were then divided into groups, allowing as far as possible litter-mate distribution among the groups and sharing males and females as evenly as possible. At 6 p.m. on the evening of operation they were given either the standard substance (III) or one of the test substances in arachis oil subcutaneously on the right flank; they were fed and watered and kept at 18—22° overnight. Early next morning they received a second injection on the left flank and the animals were then put in separate compartments of a cage and subjected to cold stress at 2—4°. The rats were examined after 3 hours at half-hourly intervals, with minimum exposure to room temperature. A control group (receiving only arachis oil) showed signs of distress and deaths after 6 ± 1½ hours. The animals were checked for absence of corneal reflex, the time of death was recorded, and the average length of survival for each group was calculated. The potency of the test substances was calculated in terms of (III) by constructing a laboratory dose-response curve (cf. Vogt, *J. Physiol.*, 1943, **102**, 341), and the potency of each compound expressed as a percentage of the standard. The faithful technical assistance of Miss M. Eleanor Cammiade in carrying out these tests is gratefully acknowledged.

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