reactions are too rapid to allow the phenol to reach the site of muconic acid formation. Phenol, however, is appreciably oxidized (10 %) to quinol and catechol and if catechol were the precursor of muconic acid, one would have expected the latter to be formed in these experiments. This suggests that neither phenol nor catechol are precursors of muconic acid in the intact animal and we are therefore inclined to the hypothesis that a reduced benzene derivative such as 1:2-dihydrobenzene-1:2-diol may be the precursor of muconic acid. In fact, the formation of this acid might well be an indication of the production of such reduced compounds *in vivo*. Our attempts to synthesize this diol in order to test this hypothesis have, so far, failed.

Most of the phenol fed was excreted in conjugated form and at the level fed (50–60 mg./kg.) the glucuronide and ethereal sulphate were excreted in approximately equal amounts. The values for ethereal sulphate (45 and 47 %; see Table 3) confirm the earlier values (36–45 %) of Williams (1938) for low levels of phenol (25–50 mg./kg.) (cf. Bray, Thorpe & White, 1952).

SUMMARY

1. Using ^{14}C -labelled benzene it has been shown in rabbits that 6–8 % of an oral dose of benzene (150–500 mg./kg.) is excreted as phenylglucuronide and 11–14 % as phenylsulphuric acid.

2. ^{14}C Phenol and ^{14}C phenylglucuronide have been prepared biosynthetically by feeding rabbits with ^{14}C benzene and ^{14}C phenol, respectively.

3. At a dose level of 50–60 mg./kg., orally administered phenol can be almost entirely accounted for as urinary metabolites in 2 days after dosing. These metabolites are phenylglucuronide (40–50 %), phenylsulphuric acid (about 45 %), quinol (about 10 %) and catechol (0.5–1.0 %).

4. Orally administered phenol does not give rise to any of the isomers of muconic acid or to phenylmercapturic acid.

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The Precipitation of 3β -Hydroxysteroids by Digitonin

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Most 3β -hydroxysteroids are precipitated by digitonin in ethanolic or methanolic solution, whilst most 3α -hydroxysteroids and steroids not carrying a hydroxyl group at C-3 are not precipitated by digitonin. This rule, originally suggested by Fernholz (1935), is subject to certain exceptions about which no simple generalizations can be made; a variety of 3β -hydroxysteroids are not precipitated in the usual conditions, and a few other steroids are precipitated. The literature up to 1949 is summarized by Fieser & Fieser (1949) (cf. also Strain, 1943). Some further exceptions to the general rule are mentioned in the discussion.

Recent work in this laboratory on the steroids of pregnant mares' urine (Brooks, Klyne, Miller & Paterson, 1952) and on the determination of the ' 3α ' and ' 3β ' ketosteroid fractions of human urine (Haslam & Klyne, 1952), led us to investigate the precipitation of 3β -hydroxysteroids by digitonin in a roughly quantitative manner by the method of Haslewood (1947a).

The results show that the configuration at C-5, and the presence or absence of substituents in the D-ring area, affect considerably the precipitability of 3β -hydroxysteroids by digitonin. A few miscellaneous experiments with 3β -acetoxysteroids and

Table 1. *Effect of varying concentration of digitonin*('Solubility product' (S) = [minimum concn. of steroid] $\times$ [concn. of digitonin] in moles²/l.²)

Digitonin concn. (before mixing, %)	Minimum final concn. of steroid for pptn. (mg./100 ml.)		S (10 ⁻⁸ moles ² /l. ²)	
	Cholesterol	Dehydro-epiandrosterone	Cholesterol	Dehydro-epiandrosterone
1.0	0.2	6.4	3.4	144
0.5	0.4	12.8	3.4	144
0.25	(0.4*) 0.8	25.6	(1.7*) 3.4	144
0.125	(0.8*) 1.6	—	(1.7*) 3.4	—

* Very slight precipitation.

with steroids not carrying a β -hydroxyl group are included; it is hoped to carry out a more thorough survey of such compounds later.

EXPERIMENTAL

Materials. Digitonin was used as supplied by British Drug Houses Ltd. or L. Light & Co. Ltd., Wraysbury, Bucks. A 1% (w/v) solution in 90% (v/v) ethanol was kept at 37° until required for use. Most of the steroids used were gifts from colleagues, to whom acknowledgements are made in Tables 2-4.

Method for examination of digitonin precipitability. The steroids were examined using a series of twofold dilutions in absolute ethanol, generally from 128 to 1 mg./100 ml. (sometimes 256 and 512 mg./100 ml. for compounds which did not form precipitates at lower concentrations). Each dilution (1 ml.) was mixed with 1% digitonin solution (4 ml.) in a stoppered tube (giving final steroid concentrations of 25.6 to 0.2 mg./100 ml., rarely 51 and 102 mg./100 ml.) and left to stand for 24 hr. at room temperature (15-20°) (cf. Haslewood, 1947*a* and unpublished data). A blank was set up with each experiment using 1 ml. of ethanol and 4 ml. of 1% digitonin solution; after 24 hr. the blank was usually clear, but occasionally it was slightly cloudy. It was considered that precipitation had occurred if a test sample was more cloudy than the blank. The concentrations given in Tables 2-4 are the minimal concentrations of steroid (in the final reaction mixture) with which precipitation occurred; approximate 'solubility products' may be calculated from these. The sign '>25.6' indicates that 25.6 mg./100 ml. was the highest concentration tested, and that no precipitation occurred.

Effect of varying concentration of digitonin. No attempt was made to carry out a thorough study of the effects of varying conditions, but two common steroids—cholesterol (cholest-5-en- β -ol) and dehydroepiandrosterone (3 β -hydroxyandrost-5-en-17-one)—were tested with 0.5, 0.25 and 0.125% as well as with 1% digitonin solutions. The results (Table 1) agreed with the expectation that

$$[\text{minimum concentration of steroid}] \times [\text{digitonin concentration}] = \text{constant.}$$

RESULTS

The results for β -hydroxy- and β -acetoxy-steroids and for a few miscellaneous compounds are collected in Tables 2-4. The results are expressed as the

minimum final concentration of steroid (c , in mg./100 ml.) which gives a precipitate in the conditions of the test.

The data of Table 1 indicate that there is an equilibrium between the digitonin-steroid complex and its components in solution, and it therefore seems permissible to calculate the 'solubility products' (S) of these complexes.

$$S = [\text{minimum concentration of steroid}] \times [\text{concentration of digitonin}].$$

Since the term 'solubility product' is generally employed with reference to ionic equilibria, its use for this non-ionic equilibrium will be distinguished by the use of quotation marks. Bergmann & Stein (1939) have used the term 'solubility product' in a similar semi-quantitative fashion for certain amino-acid complexes. Since the tests for digitonin precipitability were done only at a series of twofold dilutions, it would give a false impression of accuracy if values for the 'solubility product' (in moles²/l.²) were calculated using accurate molecular weights. It seems more appropriate to indicate the approximate 'solubility products' (for compounds of molecular weight about 300) corresponding to the various minimal concentrations of steroid (c) (see the head of Table 2).

The results of previous workers (Schönheimer & Dam, 1933; Noller, 1939; Haslewood, 1947*a*), some of which were obtained in slightly different conditions, are given in footnotes to the tables.

Separations of β -hydroxysteroids by fractional precipitation with digitonin

The results of the semi-quantitative survey suggested that it might be possible to separate certain mixtures of β -hydroxysteroids, e.g. mixtures of 5-epimers, by fractional precipitation with digitonin. Such mixtures are often formed by reduction of Δ^5 -unsaturated compounds and, whilst the isolation of the predominant 5 α -epimer is easy, the isolation of the 5 β -epimer is not (cf. Brooks, Klyne & Miller, 1953, and references there).

Mixtures of two known steroids in ethanol were taken and treated with digitonin in such conditions that the more soluble digitonide would just fail to precipitate, whilst the bulk of the less soluble digitonide would be precipitated.

In a preliminary experiment 3 β -hydroxy-5 α - (and -5 β -) androstan-17-ones were mixed in equal quantities and treated with digitonin. The precipitate (*B* fraction) and the material remaining in solution (*A* fraction) were dried and decomposed

in the normal manner with pyridine and ether (Schönheimer & Dam, 1933). The melting points of the recovered steroids showed that, as expected, the 5 β -isomer (m.p., 153–155°; less easily precipitable) was concentrated in the *A* fraction (m.p. 135–140°), whilst the 5 α -isomer (m.p., 174–175°; more easily precipitated) was concentrated in the *B* fraction (m.p. 162–170°; after recrystallization from ethyl acetate:light petroleum, m.p., 173–175°).

Table 2. *Digitonin precipitability of 3 β -hydroxysteroids*

(Minimum concentration for precipitation (*c*) is expressed as mg. steroid/100 ml. of solution after mixing with digitonin. '?' indicates very slight precipitation. References are given only for less common compounds.)

Approximate values of 'solubility products' (*S*) corresponding to given minimum concentrations of steroid for precipitation (*c*). Mol.wt. assumed 300.

<i>c</i> (mg./100 ml.)	$10^8 \times S$ (moles ² /l. ²)	<i>c</i> (mg./100 ml.)	$10^8 \times S$ (moles ² /l. ²)
0.2	4	6.4	140
0.4	8	12.8	280
0.8	16	25.6	550
1.6	35	51	1100
3.2	70	102	2200

Compound	Minimum concn. for pptn. (<i>c</i>) (mg./100 ml.)	Sources and references
Monohydroxy compounds		
5 α -Androstan-3 β -ol	0.4	<i>a</i>
Androst-5-en-3 β -ol	1.6	<i>a</i>
5 α -Cholestan-3 β -ol	0.2*†	—
Cholest-5-en-3 β -ol	0.2*†	—
5 α -Pregnan-3 β -ol	0.4	<i>b</i>
Pregn-5-en-3 β -ol	0.4	<i>b</i>
Stigmasta-5:22-dien-3 β -ol	0.2	—
5 α -Ergost-7-en-3 β -ol	0.2	<i>b</i>
5 α -Ergosta-7:22-dien-3 β -ol	0.2	<i>b</i>
5 α -Ergost-8(14)-en-3 β -ol	0.8	<i>b</i>
5 α -Ergost-14-en-3 β -ol	0.2	<i>b</i>
3-Methyl-5 α -androstan-3 β -ol	1.6	<i>c, l</i>
5 β -Cholestan-3 β -ol (coprostanol)	3.2*†	<i>d</i>
Hydroxyacids and hydroxyesters		
3 β -Hydroxychol-5-enic acid	0.8	<i>e</i>
Methyl 3 β -hydroxychol-5-enate	0.4	<i>e</i>
Methyl 3 β -hydroxy-5 α -cholanate	0.4	<i>b</i>
Methyl 3 β -hydroxy-5 α -etianate	0.4	<i>b</i>
Methyl 3 β -hydroxy-eti-5-enate	0.4	<i>b</i>
Hydroxyketones		
3 β -Hydroxy-5 α -cholestan-6-one	0.4	—
3 β -Hydroxy-5 α -cholestan-7-one	0.4	—
3 β -Hydroxycholest-5-en-7-one	0.4	—
3 β -Hydroxy-5 α -androstan-7-one	0.8	<i>a, m</i>
3 β -Hydroxy-5 α -androstan-17-one	6.4	<i>f</i>
3 β -Hydroxyandrost-5-en-17-one	6.4	<i>f</i>
3 β -Hydroxy-5 α -pregnan-20-one	0.2	<i>g</i>
3 β -Hydroxypregn-5-en-20-one	0.8	<i>e</i>
3 β -Hydroxy-5 α -D-homoandrostan-17a-one	1.6	—
3 β -Hydroxy-D-homoandrost-5-en-17a-one	3.2	<i>h, n</i>
3 β -Hydroxy-5 α -des-D-androstan-14-one	26	<i>i, o</i>
3 β -Hydroxy-des-D-androst-5-en-14-one	26	<i>i, p</i>
3 β -Hydroxy-5 β -androstan-17-one	>51?	<i>i</i>
3 β -Hydroxy-5 β -pregnan-20-one	51	<i>j</i>
Sapogenins		
5 α :22a-Spirostan-3 β -ol (tigogenin)	3.2†	<i>j</i>
22a-Spirost-5-en-3 β -ol (diosgenin)	3.2	<i>j</i>
5 β :22b-Spirostan-3 β -ol (sarsasapogenin)	12.8†	<i>j</i>

Table 2 (cont.)

Compound	Minimum concn. for pptn. (c) (mg./100 ml.)	Sources and references
Dihydroxy compounds		
Cholest-5-ene-3 β :4 β -diol	3.2	—
5 α -Androstane-3 β :17 β -diol	26	<i>f</i>
Androst-5-ene-3 β :17 α -diol	26	<i>i</i>
Androst-5-ene-3 β :17 β -diol	51	<i>i</i>
5 α :14 β -Androstane-3 β :17 α -diol	26	<i>a, q</i>
5 α -Pregnane-3 β :20 α -diol	0.8	—
5 α -Pregnane-3 β :20 β -diol	6.4	—
Pregn-5-ene-3 β :20 α -diol	3.2	—
Pregn-5-ene-3 β :20 β -diol	12.8	—
5 α -Pregn-16-ene-3 β :20 β -diol	1.6	<i>j, r</i>
Pregna-5:16-diene-3 β :20 α -diol	3.2	<i>k, s</i>
Pregna-5:16-diene-3 β :20 β -diol	3.2	<i>j, r, t</i>
5 α -D-Homoandrostane-3 β :17 $\alpha\beta$ -diol	3.2	<i>h</i>
17 α -Methyl-5 α -D-homoandrostane-3 β :17 $\alpha\beta$ -diol (uranediol)	26	—
17 α -Ethinylandroster-5-ene-3 β :17 β -diol	>51	<i>i</i>
5 β -Pregnane-3 β :20 β -diol	>51	<i>j</i>
Dihydroxyketones		
3 β :14 α -Dihydroxy-5 α -androstane-17-one	>26	<i>a, q</i>
3 β :14 α -Dihydroxyandrost-5-en-17-one	>26	<i>a, q</i>
3 β :17 α -Dihydroxy-5 α -pregnan-20-one	0.4	<i>j</i>

* Haslewood (1947*a*) found that these three compounds were precipitated at minimum concentrations of <0.2, <0.2 and 6 mg./100 ml. respectively.

† Schönheimer & Dam (1933) gave values for the solubilities of the digitonides of these compounds (in 96% v/v, ethanol) from which the following solubility products are calculated: $10^8 \times S = 0.9$, 1.5, 18 moles²/l.².

‡ Noller (1939) gave the solubility products S (in moles²/l.²) for these compounds as 16×10^{-8} and 970×10^{-8} respectively.

Sources (in some cases the compounds used were prepared from starting materials provided by the following workers): *a*, Dr H. B. MacPhillamy, CIBA Pharmaceuticals Inc., Summit, N.J.; *b*, Dr D. H. R. Barton, Birkbeck College, London; *c*, Prof. V. Prelog, Eidg. Technische Hochschule, Zürich; *d*, Prof. G. A. D. Haslewood, Guy's Hospital Medical School, London; *e*, Dr W. L. C. Veer, N. V. Organon, Oss, Netherlands; *f*, Dr V. Petrow, British Drug Houses Ltd., London; *g*, Dr J. A. Moore, Parke Davis and Co., Detroit, Michigan; *h*, Dr H. Heusser, Eidg. Technische Hochschule, Zürich; *i*, Dr A. Wettstein, CIBA A.G., Basel; *j*, Dr C. Djerassi and Dr G. Rosenkranz, Syntex S.A., Mexico City, and Wayne University, Detroit, Michigan; *k*, Prof. A. Ercoli, Laboratori Ricerche Vister, Casatenovo Brianza, Como, Italy.

References: *l*, Ruzicka, Meister & Prelog (1947); *m*, Heard & McKay (1946); *n*, Heusser, Herzig, Fürst & Plattner (1950); *o*, Achtermann (1934); *p*, Köster & Logemann (1940); *q*, St André, MacPhillamy, Nelson, Shabica & Scholz (1952); *r*, Marker *et al.* (1941); *s*, Ercoli & de Ruggieri (1952); *t*, Butenandt & Schmidt-Thomé (1939).

Table 4. Digitonin precipitability; miscellaneous compounds

(Concentrations and references as in Table 2.)

(Concentrations and references as in Table 2.)			Minimum concn. for pptn. (c) (mg./100 ml.)	Sources and references
Compound	Minimum concn. for pptn. (c) (mg./100 ml.)	Sources		
3β-Acetoxy derivative of				
5 α -Cholestan-3 α -ol	51	—	102*	<i>b</i>
Cholest-5-ene	>51	—	>102	<i>b</i>
5 α -Pregnane	51	<i>b</i>	51	<i>c, l</i>
Methyl 5 α -cholanate	>26	<i>b</i>	>102	<i>e</i>
Androst-5-en-17-one	>51	<i>f</i>	5 α -Cholestan-2 α -ol	>102
5 α -Androstan-17-one	>102	<i>f</i>	5 α -Cholestan-2 β -ol	51
5 α -D-Homoandrostane-17 α -one	>51	—	5 α -A-Norandrostane-2 α -ol	102
5 α -Pregnan-20-one	26	<i>g</i>	5 α -A-Norandrostane-2 β -ol	12.8
Pregn-5-en-20-one	26	<i>e</i>	5 α -Cholestan-3-one	6.4†
5 α -Pregn-16-en-20-one	26	<i>j</i>	Cholest-4-en-3-one	>102†
5 β -Pregnan-20-one	>51	<i>j</i>		—

* Noller (1939) found the solubility product of this compound to be 940×10^{-8} moles²/l.².

† Haslewood (1947*a*) found that these two compounds were precipitated at minimum concentrations of 60 and 350 mg./100 ml. respectively.

References: *u*, Ruzicka, Plattner & Furrer (1944); *v*, Ruzicka, Prelog & Meister (1945).

Table 5. Separation of 3 β -hydroxysteroids with digitonin

(In the androstane series the two compounds were dissolved in ethanol (35 ml.) and treated with digitonin (1.40 g.) in 90% (v/v) ethanol (140 ml.). After standing 24 hr. at room temperature the precipitate (*B*-fraction) was centrifuged and washed once with its own volume of 92% ethanol, the washings being added to the supernatant (*A* fraction). Similar proportions were used in the pregnane series.)

Mixture taken	Androstane series (3 β -Hydroxy-17-ketones)		Pregnane series (3 β -Hydroxy-20-ketones)	
	5 β -Compound	Δ^5 -Compound	5 β -Compound	Δ^5 -Compound
Wt. (mg.)	70	70	43	43
M.p. (°)	153–155	152–154	143–144	191–194
$[\alpha]_D$ in EtOH	+89°	+10°	+101°	+25°
Fractions separated				
	<i>A</i>	<i>B</i>	<i>A</i>	<i>B</i>
Wt. (mg.)	56	84	36	48
M.p. (°)	153–156	105–135	134–138	165–183
Content of Δ^5 -compound (%)				
By Allen† reaction	10	72	—	—
By optical rotation	13	82	26	88
Product after recrystallization				
M.p.	152–154	134–140*	141–142	185–189

* This compound sometimes crystallizes in needles, m.p. 140–141°.

† Allen, Hayward & Pinto (1950).

No simple method exists for determining the proportions of 5 α - and 5 β -isomers in a mixture; further experiments were therefore carried out with mixtures of 5 β -compounds (less precipitable) and the related Δ^5 -unsaturated compounds (more precipitable), since the proportions of unsaturated material in the two fractions can be determined by optical-rotation measurements and, for 17-ketones of the androstane series, by the sulphuric acid colour reaction of Allen, Hayward & Pinto (1950).

The results (Table 5) show that the less precipitable (5 β) compounds can easily be obtained pure from the *A* fractions after one crystallization. The more precipitable (Δ^5) compounds are largely contained in the *B* fraction, but are contaminated with 5 β -compound even after recrystallization.

DISCUSSION

3 β -Hydroxysteroids

5 α - and 5 β -Epimers. The most important point which emerges from this work is the fact that 3 β -hydroxysteroids of the 5 β -series are precipitated by digitonin only at much higher concentrations than those which are minimal for the corresponding compounds of the 5 α -series. The differences are such that digitonin precipitation in controlled conditions may be used to separate 5 α - and 5 β -isomers. Such isomers are difficult to separate by chromatography.

Haslewood (1947*b*) found that whilst 5 α -cholestan-3-one (cholestanone), cholest-4-en-3-one and cholest-5-en-3-one in high concentrations were precipitated by digitonin, 5 β -cholestan-3-one (coprostanone) was not. He suggested that *cis*-union of

rings *A* and *B* might be less favourable to digitonide formation than the flat configuration in *trans*-, Δ^4 -, or Δ^5 -compounds. This seems a reasonable hypothesis, especially in view of what is now known regarding the preferred conformations of 5 β (*A/B cis*) and 5 α (*A/B trans*) steroids (Barton, 1950, 1953). The present experimental work supports this suggestion.

Olefinic linkages. Olefinic linkages at C-5, C-7, C-8 or C-14 usually make no appreciable difference to the digitonin precipitability of 3 β -hydroxy compounds. Androst-5-en-3 β -ol and some of the pregn-5-ene-3:20-diols are exceptions. Hirschmann (1943) found that whilst 5 α -androstane-3 β :16 α :17 β -triol was readily precipitated with digitonin, the corresponding Δ^5 -compound was only partially precipitated.

Side chains. Different hydrocarbon, acid or ester side chains at C-17, in the normal 17 β -configuration, usually make no appreciable difference to the digitonin precipitability. Fieser & Fieser (1949) give references to a number of compounds carrying 17 α side chains which are not precipitable. The spiroketal side chain of the sapogenins reduces the digitonin precipitability greatly.

'Lanosterol' (lanostadienol), which is now known to be 4:4:14-trimethylcholesta-8(9):24-dien-3 β -ol (Voser, Mijović, Heusser, Jeger & Ruzicka, 1952) gives a sparingly soluble digitonide (Ruzicka, Denss & Jeger, 1945). 'Dihydrolanosterol' (lanostenol), in which the 24:25 double bond is saturated, gives a digitonide which is relatively easily soluble.

Keto groups. Ketone groups at C-6, C-7 and C-20 make little or no difference to the digitonin precipitability (except in the case of 5 β -pregnane-3 β -

ol-20-one, which is much less easily precipitable than 5β -cholestan- 3β -ol. Ketone groups at C-17 (and at C-17a in the *D*-homosteroid series) reduce the digitonin precipitability considerably. The two des-*D*-14-ketones were precipitated only at high concentrations. (These are steroids lacking ring *D*; for nomenclature see *J. chem. Soc.*, 1952, p. 5060.)

Additional hydroxyl groups in positions other than C-3. Hydroxyl groups at C-20 reduce the digitonin precipitability somewhat (20β -hydroxyl more than 20α -), and hydroxyl groups at C-17 reduce it even more so. Butenandt, Tscherning & Hanisch (1935) found that 5α -androstane- 3β : 17β -diol was not precipitable by digitonin. Hirschmann & Hirschmann (1950) stated that the corresponding Δ^5 -compound gave a rather soluble digitonide. (Both these compounds were called 17α by the original authors.) The pregnanetriol of pregnant mares' urine (5α -pregnane- 3β : 16α : 20β -triol; Haslewood, Marrian & Smith, 1934) and some related compounds (Hirschmann, Hirschmann & Daus, 1949) are not precipitated by digitonin.

Marker, Rohrmann & Wittle (1938) isolated uranediol (17α -methyl-*D*-homoandrostane- 3β : $17\alpha\beta$ -diol) from pregnant mares' urine by a method involving fractional precipitation with digitonin. Brooks *et al.* (1952), using the same method, obtained uranediol, 5α -pregnane- 3β : 20β -diol, and 5α -pregnane- 3β : 20α -diol from the ' β 2' fraction (more soluble digitonides).

3\beta-Acetoxy compounds

3β -Acetoxy- 5α -pregnan-20-one is precipitable by digitonin (Butenandt & Mamoli, 1935), although it has no free 3β -hydroxyl group. This fact was used by Butenandt and Mamoli and by Shoppee (1949) in separating this compound from its 17α -isomer. Whitman & Schwenk (1940) claimed that precipitation of 3β -acetoxy- 5α -pregn-5-en-20-one by digitonin could be used to separate this compound from other steroid acetates.

We have found that some other 3β -acetoxy compounds are precipitated at high concentrations (Table 3).

Miscellaneous compounds

Cholestan- 2β -ol (Ruzicka, Plattner & Furrer, 1944; configuration proved by Fürst & Plattner, 1949) was precipitated at high concentration; its 2α -epimer could not be precipitated at the highest concentration tested. The *A*-norandrostane- $2'\beta'$ -ol (Ruzicka, Prelog & Meister, 1945) and $3'\alpha'$ -methyl-androstan- $3'\beta'$ -ol (Ruzicka, Meister & Prelog, 1947), configurations of which were allotted on the basis of their digitonin precipitability, were precipitated at concentrations appreciably lower than their $2'\alpha'$ - and $3'\alpha'$ -hydroxy-epimers.

Some recent examples of compounds other than 3β -hydroxysteroids which are precipitated by digitonin are as follows: 5α -pregn-2-en-20-one (Lieberman, Dobriner, Hill, Fieser & Rhoads, 1948); 3β -aminocholestane (Dodgson & Haworth, 1952) (the amino group almost certainly has the β (equatorial) configuration, since the compound was obtained by reducing an oxime with sodium and amyl alcohol (cf. Barton, 1953)); conessine (Bertho, 1950), shown by Haworth, McKenna & Whitfield (1953) to be a 3β -dimethylaminopregn-5-ene derivative.

SUMMARY

1. The precipitation of 3β -hydroxysteroids by digitonin has been studied in a roughly quantitative manner by the method of Haslewood (1947a).

2. 3β -Hydroxysteroids of the 5α -(*A/B-trans*) series and of the Δ^5 -unsaturated series are precipitated by digitonin in much higher dilution than are the corresponding steroids of the 5β -(*A/B-cis*) series. This difference in behaviour with digitonin can be used in the separation of such steroids.

3. The behaviour of some 3β -acetoxy steroids and other compounds with digitonin has been studied.

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The Lysing Action of Enzymes on a Sample of Mycelium of *Penicillium griseofulvum* Dierckx

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Soil organic matter is composed, at least in part, of living micro-organisms and those parts of their remains which are resistant to chemical and enzymic decomposition. A knowledge of the action of enzymes on fungal mycelium may perhaps give some idea of the composition of this resistant fraction. An examination has, therefore, been made of the breakdown by enzymic action of a sample of mycelium of *Penicillium griseofulvum* Dierckx, the composition of which has been studied by Smithies (1952).

The effect of autolysis on the composition of mould mycelium and the nature of the products liberated have been investigated (e.g. by Woolley & Peterson, 1937) usually with special regard to the protein constituent and the production of amino acids, or the yield of a particular metabolic product.

An interesting point is mentioned by Foster (1949) that, whereas in certain bacteria and actinomycetes the phenomenon of self digestion is well known, this does not occur amongst the filamentous fungi, and there remains an enzyme-resistant portion.

This paper deals with the liberation and breakdown of the carbohydrate, protein and chitin con-

stituents of the mycelium, both on autolysis of the air-dried mycelium and on treatment with enzymes from other sources.

MATERIALS AND GENERAL METHODS

Unless otherwise stated, these were as given by Smithies (1952).

Protein. This was estimated by hydrolysis of mycelium with 6N-HCl and determination of α -amino-acid N by the ninhydrin- CO_2 titration method of Van Slyke, MacFadyen & Hamilton (1941), which represents about 72% of the total protein N (Smithies, 1952).

Chitin. This was estimated in mycelium samples by determination of glucosamine hydrochloride in 6N-HCl hydrolysates (Smithies, 1952).

Carbohydrate. This was determined in mycelium by first taking up the sample in 72% (w/w) H_2SO_4 and applying the orcinol method of Pirie (1936) as used by Smithies (1952).

Snail enzyme. This was obtained from the crops and guts of starved snails (*Helix aspersa*) as described by Bawden & Pirie (1946).

Trypsin. Suspensions of dry preparations from pancreas were used as described by Holden & Tracey (1950).

Papain. This was prepared and activated as by Tracey (1948).