Studies in Detoxication

59. THE METABOLISM OF ALKYLBENZENES. THE BIOLOGICAL REDUCTION OF KETONES DERIVED FROM ALKYLBENZENES

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(*Received* 9 *December* 1953)

Recently we showed that acetophenone was asymmetrically reduced in rabbits to (−)-methylphenylcarbinol (Smith, Smithies & Williams, 1954). Five more ketones, namely, ethyl phenyl, phenyl propyl, benzyl methyl, benzyl ethyl, and methyl phenethyl ketones have now been studied, and it appears that some of these are also asymmetrically reduced in the rabbit. The glucuronides (glucosiduronic acids) isolated in this work were needed as reference compounds for other work in progress on alkylbenzenes.

EXPERIMENTAL

Melting points are corrected and optical rotations are for 1% solutions in chloroform, the error being ±1°.

Isolation of glucuronides

From ethyl phenyl ketone. Propiophenone (b.p. 218°; 2·5 g.) was fed to each of four rabbits. The urine was collected for 24 hr. and the glucuronide gum (10·3 g.) isolated through its basic lead salt. This gum was methylated and acetylated (cf. Kamil, Smith & Williams, 1951) and the crude triacetyl methyl ester (9·2 g.) had $[\alpha]_D$ −72°. Recrystallization from methylated spirit yielded 4·91 g. of white needles with $[\alpha]_D^{23}$ −80°. The rotation remained unchanged on further crystallization and the pure *methyl*((−)-*ethylphenylcarbinyl tri*-O-*acetyl*-β-D-*glucosid*)*uronate* had m.p. 127° and $[\alpha]_D^{20}$ −80°. (Found: C, 58·5; H, 6·3. $C_{22}H_{28}O_{10}$ requires C, 58·3; H, 6·2%.) Examination of the mother liquors from this ester did not reveal the presence of the (+)-isomer.

From phenyl n-*propyl ketone.* Butyrophenone (b.p. 232°; 2 g.) was fed to each of six rabbits and from the 24 hr. urine 12·2 g. of glucuronide gum were isolated. Part of this (7 g.) was converted into a gummy triacetyl methyl ester (5·75 g.; $[\alpha]_D^{20}$ −63°). This ester was eventually crystallized with great difficulty from 50% (v/v) aqueous acetic acid by allowing the solution to evaporate slowly. *Methyl* ((−)-*phenyl*-n-*propylcarbinyl tri*-O-*acetyl*-β-D-*glucosid*)*uronate* (1·84 g.) was obtained as colourless needles, m.p. 104° (from aqueous ethanol) and $[\alpha]_D^{20}$ −78·8°. (Found: C, 59·0; H, 6·7. $C_{23}H_{30}O_{10}$ requires C, 59·2; H, 6·5%.) Further examination of the mother liquors from this ester failed to reveal the presence of the (+)-isomer.

From benzyl methyl ketone. The ketone (b.p. 214°; 2·25 g.) was fed to each of four rabbits and from the 24 hr. urine, 12 g. of glucuronide gum were isolated. This yielded 10·3 g. of crude triacetyl methyl ester with $[\alpha]_D$ −21·2°. Purification of this ester from ethanol and *iso*propyl ether eventually yielded 4·76 g. of pure *methyl* ((+)-*benzylmethylcarbinyl tri*-O-*acetyl*-β-D-*glucosid*)*uronate*, as needles m.p. 118° and $[\alpha]_D^{20}$ −24°. (Found: C, 58·35; H, 6·4. $C_{22}H_{28}O_{10}$ requires C, 58·35; H, 6·2%.) The mother liquors from this ester did not appear to contain any of the (−)-isomer.

From (±)-*benzylmethylcarbinol.* This carbinol, b.p. 105°/20 mm., was prepared in 80% yield by reducing the ketone with lithium aluminium hydride (Brown, 1951). Quantitative estimation of the glucuronic acid output after feeding the carbinol (0·5 g./kg.) to three animals showed that about 43% was excreted as conjugated glucuronic acid by rabbits. There was also a small rise (corresponding to about 3% of the dose) in excretion of ethereal sulphates, suggesting a slight nuclear oxidation. The glucuronides of both isomers of benzylmethylcarbinol were isolated after feeding the carbinol.

The (±)-carbinol was fed to six rabbits (2·5 g. each). The gummy triacetyl methyl ester was prepared in the usual manner and repeated recrystallization from 50% aqueous ethanol yielded methyl ((+)-benzylmethylcarbinyl tri-*O*-acetyl-β-D-glucosid)uronate (2·4 g.), m.p. 120° and $[\alpha]_D^{20}$ −24°. (Found: C, 58·7; H, 6·5%.) This ester did not depress the m.p. of the glucuronide obtained by feeding benzyl methyl ketone (see above).

The (−)-isomer was obtained in another experiment. (±)-Benzylmethylcarbinol was fed to seven rabbits (2 g. each). From the 24 hr. urine 14·2 g. of glucuronide gum was obtained and this yielded 11 g. of crude triacetyl methyl ester with $[\alpha]_D^{22}$ −30°. Three recrystallizations from ethanol yielded material with decreased rotation ($[\alpha]_D$ −26°), and from the combined mother liquors after evaporation, 2·2 g. of material with $[\alpha]_D$ −34° was obtained. Four recrystallizations of the latter material from *iso*propyl ether gave 0·67 g. of crystals with $[\alpha]_D^{25}$ −46° and m.p. 116°. Further recrystallizations from 80% ethanol or *iso*propyl ether raised the m.p. but the rotation remained constant. Finally, the *methyl* ((−)-*benzylmethylcarbinyl tri*-O-*acetyl*-β-D-*glucosid*)*uronate* (0·35 g.) was obtained as colourless needles, m.p. 127·5° and $[\alpha]_D^{25}$ −46°. (Found: C, 58·3; H, 6·2. $C_{22}H_{28}O_{10}$ requires C, 58·35; H, 6·2%.)

From methyl phenethyl ketone. The ketone (b.p. 234°), prepared as described by Vogel (1948), was fed to four rabbits (2 g. each) and the urine (500 ml.) collected during 24 hr. The urine gave a strong naphthoresorcinol reaction and slightly reduced Benedict's reagent but not Fehling's solution. It gave a positive iodoform test for methyl ketone and methyl carbinol and a slight positive test with Brady's reagent for ketones. It yielded 5·5 g. of glucuronide gum and 4·8 g. of crude amorphous triacetyl methyl ester with $[\alpha]_D$ −33°. This ester crystallized with difficulty from

aqueous methanol yielding 0·85 g. of semi-crystalline solid with $[\alpha]_D$ – 26·5°. Evaporation of the mother liquors yielded a gum with $[\alpha]_D$ – 37° which resisted all attempts at crystallization. The solid material on recrystallization from aqueous methanol yielded 0·1 g. of colourless needles melting sharply at 120° and $[\alpha]_D^{20}$ – 27°. (Found: C, 59·1; H, 6·6. $C_{23}H_{30}O_{10}$ requires C, 59·2; H, 6·5%.) This is probably *methyl((+)-methylphenethylcarbinyl tri-O-acetyl-β-D-glucosid)uronate.*

From benzyl ethyl ketone. The ketone (10 g., b.p. 225°), prepared according to Blatt (1943), was fed to four rabbits and the urine collected for 48 hr. The naphthoresorcinol reaction was positive, but Fehling's, Benedict's and Brady's reagents gave negative tests. The urine yielded 7·2 g. of glucuronide gum and 5·6 g. of gummy triacetyl methyl ester with $[\alpha]_D^{23}$ – 27·5°. A solution of the ester in ethanol slowly deposited crystals (0·16 g. with $[\alpha]_D^{23}$ – 19·5° and a further crop of 0·13 g. with $[\alpha]_D$ – 22°). Recrystallization of the first crop from *iso*propyl ether gave 90 mg. of fine needles, m.p. 113° and $[\alpha]_D^{23}$ – 22°. (Found: C, 59·15; H, 6·7. $C_{23}H_{30}O_{10}$ requires C, 59·2; H, 6·5%.) This compound is probably *methyl((+)-benzylethylcarbinyl tri-O-acetyl-β-D-glucosid)uronate.* The residual gum from these crystals had a higher negative rotation than this ester, and had not crystallized after several months.

DISCUSSION

Thierfelder & Daiber (1923) fed a number of mixed ketones to rabbits and isolated the glucuronides formed as metallic salts. The molecular rotations of these compounds are quoted in Table 1. These workers made no specific comment on the stereochemistry of the glucuronides. Recently, Smith *et al.* (1954) have shown that acetophenone is converted in rabbits into the glucuronide of (–)-methylphenylcarbinol. An examination of the rotations of the glucuronides given in Table 1 suggests that propiophenone and butyrophenone, like acetophenone, are converted in rabbits into the glucuronides of the corresponding (–)-carbinols. Benzyl *iso*propyl ketone, however, produced a glucuronide whose rotation was much less than the other three and it is possible that in this case a (+)-carbinol is produced (cf. benzyl methyl ketone, below).

In the present work the glucuronides obtained after feeding the ketones have been characterized as triacetyl methyl esters, whose rotations are quoted in Table 2, from which it appears that propio- and butyro-phenones are reduced in rabbits to carbinols of the same sign of rotation as (–)-methylphenylcarbinol, since the molecular rotations of the triacetyl methyl esters of the glucuronides are almost identical. (–)-Ethylphenylcarbinol and (–)-phenyl-*n*-propylcarbinol on rotation arguments have the same relative configuration as (–)-methylphenylcarbinol (Freudenberg, 1932). It thus appears that aceto-, propio- and butyrophenones are asymmetrically reduced in rabbits to carbinols configuratively related to L-(–)-glyceraldehyde.

With benzyl methyl ketone, however, the triacetyl methyl ester of the glucuronide has a low

Table 1. *Glucuronides isolated by Thierfelder & Daiber* (1923) *after feeding certain ketones to rabbits*

Ketone fed	Formula of glucuronide	$[\alpha]_D$ in water (°)	$[M]_D$ (°)
$Ph.CO.CH_3$	$C_{14}H_{17}O_7K$, $1·5H_2O$	–124	–450
$Ph.CO.CH_2.CH_3$	$C_{15}H_{19}O_7K$	–126	–441
$Ph.CO.CH_2.CH_2.CH_3$	$(C_{16}H_{21}O_7)_2Cd$	–109	–415
$Ph.CH_2.CO.CH(CH_3)_2$	$C_{17}H_{23}O_7K$	–55	–208

Table 2. *Optical rotations of the triacetyl methyl esters of glucuronides isolated after feeding certain ketones to rabbits*

Compound fed	Carbinol present in glucuronide	Rotation (*c*, 1 in $CHCl_3$) of triacetyl methyl ester of glucuronide of carbinol: $[\alpha]_D$ (°)	$[M]_D$ (°)
$Ph.CO.CH_3$	(–)-$Ph.CHOH.CH_3$	–83*	–364
(±)-$Ph.CHOH.CH_3$	(–)-$Ph.CHOH.CH_3$	–83*	–364
	(+)-$Ph.CHOH.CH_3$	–15*	–66
$Ph.CO.CH_2.CH_3$	(–)-$Ph\ CHOH.CH_2.CH_3$	–80	–362
$Ph.CO.CH_2.CH_2.CH_3$	(–)-$Ph.CHOH.CH_2.CH_2.CH_3$	–79	–368
$Ph.CH_2.CO.CH_3$	(+)-$Ph.CH_2.CHOH.CH_3$	–24	–108
(±)-$Ph.CH_2.CHOH.CH_3$	(+)-$Ph.CH_2.CHOH.CH_3$	–24	–108
	(–)-$Ph.CH_2.CHOH.CH_3$	–46	–208
$Ph.CH_2.CO.CH_2.CH_3$	(+)-$Ph.CH_2.CHOH.CH_2.CH_3$†	–22	–103
$Ph.CH_2.CH_2.CO.CH_3$	(+)-$Ph.CH_2.CH_2.CHOH.CH_3$†	–27	–126

* From Smith *et al.* (1954). † See text.

$[\alpha]_D$ of $-24°$. This glucuronide could be derived from (+) or (−)-benzylmethylcarbinol. (±)-Benzylmethylcarbinol was therefore fed, and the expected two diastereoisomeric glucuronides were isolated as triacetyl methyl esters, one with $[\alpha]_D - 24°$ identical with the compound isolated after feeding the ketone, and the other with $[\alpha]_D - 46°$. The glucuronide with the higher negative rotation was obviously derived from (−)-benzylmethylcarbinol and the other from the (+)-isomer. Thus it was clear that benzyl methyl ketone was reduced in the rabbit to (+)-benzylmethylcarbinol. (+)-Benzylmethylcarbinol has been correlated chemically with L-(−)-glyceraldehyde (Levene & Walti, 1931; see also Freudenberg, 1932). Thus all the four ketones considered so far appear to be asymmetrically reduced in the rabbit to secondary carbinols related to L-(−)-glyceraldehyde.

With the other two ketones, methyl phenethyl ketone and benzyl ethyl ketone, similar conclusions cannot be drawn, since the yields of crystalline triacetyl methyl ester isolated were very small compared with the amount of non-crystalline ester actually obtained. Furthermore, the rotation of the crystalline ester was less negative than that of the bulk of material from which it was isolated although the differences were not very great. Thus with methyl phenethyl ketone, 0·85 g. of crystalline ester with $[\alpha]_D - 26·5°$ was separated from 4·8 g. of non-crystalline ester with $[\alpha]_D - 33°$, whereas with benzyl ethyl ketone, 0·16 g. of crystalline ester, $[\alpha]_D - 19·5°$ came from 5·6 g. of non-crystalline ester, $[\alpha]_D - 27·5°$. The non-crystalline esters could have contained the other diastereoisomer but this could not be proved since they failed to crystallize. (Other metabolites of these ketones are probably present and complicate matters.) Methyl phenethyl ketone is reported to yield phenaceturic acid in the dog (Dakin, 1908–9; Hermanns, 1913). Suggestions as to the stereochemical nature of these crystalline esters, however, can be made. The crystalline methyl (methylphenethylcarbinyl triacetyl glucosid)uronate, $[M]_D - 126°$, and methyl (benzylethylcarbinyl triacetyl glucosid)uronate, $[M]_D - 103°$, have rotations very similar to that of methyl ((+)-benzylmethylcarbinyl triacetyl glucosid)uronate, $[M]_D - 108°$. The $[M]_D$ of the (−)-form of the last ester is $-208°$. Furthermore, according to Freudenberg (1932), the corresponding optical forms of the configuratively related carbinols

$Ph.CH_2.CHOH.CH_3$ ($[M]_D$ 38·5°),

$Ph.CH_2.CHOH.C_2H_5$ ($[M]_D$ 51·5°)

and $Ph.CH_2.CH_2.CHOH.C_2H_5$ ($[M]_D$ 31°)

have the same sign of rotation, the (+)-forms being related to (−)-methylphenylcarbinol ($Ph.CHOH.CH_3$). On these grounds it is suggested that in methyl (methylphenethylcarbinyl triacetyl glucosid)uronate, m.p. 120°, and methyl (benzylethylcarbinyl triacetyl glucosid)uronate, m.p. 113°, the aglycones are in the (+)-form.

SUMMARY

1. Ethyl phenyl, phenyl *n*-propyl, benzyl methyl, benzyl ethyl and methyl phenethyl ketones are partly reduced in the rabbit to secondary carbinols which are excreted conjugated with glucuronic acid. The glucuronides have been isolated and characterized as triacetyl methyl esters.

2. Ethyl phenyl and phenyl *n*-propyl ketones appear to be reduced to the corresponding (−)-carbinols and benzyl methyl ketone to the (+)-carbinol. These carbinols are configuratively related to (−)-methylphenylcarbinol.

3. (±)-Benzylmethylcarbinol gives rise in rabbits to the glucuronides of both isomers of the carbinol.

The expenses of this work were partly defrayed by a grant from the Medical Research Council. One of us (R.H.S.) is grateful to the Council for a Scholarship.

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