REFERENCES

Baer, E. & McArthur, C. S. (1944). *J. biol. Chem.* **154**, 451.
Chargaff, E., Ziff, M. & Rittenberg, D. (1942). *J. biol. Chem.* **144**, 343.
Entenman, C., Taurog, A. & Chaikoff, I. L. (1944). *J. biol. Chem.* **155**, 13.
Folch, J. (1942). *J. biol. Chem.* **146**, 35.
Folch, J., Schneider, H. A. & Van Slyke, D. D. (1940). *J. biol. Chem.* **133**, xxxiii.
Fowden, L. (1951). *Biochem. J.* **48**, 327.
Hack, M. H. (1953). *Biochem. J.* **54**, 602.
Hanahan, D. J., Turner, M. B. & Jayko, M. E. (1951). *J. biol. Chem.* **192**, 623.
Hecht, E. & Mink, C. (1952). *Biochim. biophys. Acta*, **8**, 641.
Kirk, E., Page, I. H. & Van Slyke, D. D. (1934). *J. biol. Chem.* **106**, 203.
Kline, L., Gegg, J. E. & Sonoda, T. T. (1951). *Food Tech., Champaign*, **5**, 181.
Lea, C. H., Hannan, R. S. & Rhodes, D. N. (1951). *Biochim. biophys. Acta*, **7**, 366.
Lea, C. H. & Rhodes, D. N. (1953). *Biochem. J.* **54**, 467.
Levine, C. & Chargaff, E. (1951). *J. biol. Chem.* **192**, 465.
Moore, S. & Stein, W. H. (1948). *J. biol. Chem.* **176**, 367.
Outhouse, E. L. (1937). *Biochem. J.* **31**, 1459.
Schmidt, G., Hershman, B. & Thannhauser, S. J. (1945). *J. biol. Chem.* **161**, 523.
Taylor, T. W. J. & Baker, W. (1937). *Sidgwick's Organic Chemistry of Nitrogen*, 2nd ed., p. 172. Oxford: Clarendon Press.

Studies in Detoxication

58. THE METABOLISM OF ALIPHATIC ALCOHOLS. THE GLUCURONIC ACID CONJUGATION OF CHLORINATED AND SOME UNSATURATED ALCOHOLS

BY J. N. SMITH AND R. T. WILLIAMS
Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2

(*Received* 4 *November* 1953)

Kamil, Smith & Williams (1953*a*) have shown that secondary and tertiary aliphatic alcohols are excreted by rabbits largely conjugated with glucuronic acid, and within the group of compounds studied, the extent of this conjugation increases with the number of carbon atoms. With primary alcohols, on the other hand, the conjugation is low. In general, the extent of conjugation increases in the order primary, secondary, tertiary. The reverse order appears to hold for the extent of oxidation of the alcohols *in vivo*. Although the pharmacological activity of alcohols depends on a number of factors, it does appear that, within an isomeric series, hypnotic activity increases as one passes from primary through secondary to tertiary alcohols (cf. Burger, 1951). In fact, the only simple alcohol which has found therapeutic use besides ethanol is tertiary pentanol (amylene hydrate). There thus appears to be a possible correlation between extent of glucuronic acid conjugation and hypnotic activity.

The introduction of halogens into organic compounds by substitution is often looked upon as a reasonable means of increasing pharmacological activity and, in the alcohol series, 2:2:2-trichloroethanol, 1:1:1-trichloro*iso*propanol (Isopral) and 1:1:1-trichloro-*tert.*-butanol (chloretone, chlorbutol) are much more powerful in their anaesthetic and hypnotic effects than the corresponding unsubstituted alcohols. It would therefore be expected that these substituted alcohols would be largely conjugated with glucuronic acid. The glucuronic acid conjugation of a number of chlorinated alcohols has therefore been measured and compared with that of the corresponding unsubstituted alcohols. Trichloroethanol is an active narcotic (Lehmann & Knoefel, 1938; 1939; Butler, 1948), and Butler (1949) has already shown that it is highly conjugated with glucuronic acid in the dog.

Primary alcohols are usually rapidly oxidized *in vivo* and their glucuronic acid conjugation is very low (Kamil *et al.* 1953*a*, *b*). Owing to the reported high conjugation of trichloroethanol, it was therefore of interest to study the effect upon glucuronic acid conjugation of progressive substitution of the β-carbon of ethanol with chlorine.

Recently the acetylenic tertiary alcohol, 3-methylpent-1-yn-3-ol (Oblivon, Dormison) has been recommended as a mild hypnotic, and it was of interest to include this in our studies. Acetylene itself was once used as a surgical anaesthetic, and Bock (1930) reported both diethylethynylcarbinol and *tert.*-butylethynylmethylcarbinol to produce sleep in dogs in doses of 0·2–0·25 g./kg. body wt. In order to assess the effect of the triple bond on the conjugation, the corresponding saturated alcohol, 3-methylpentan-3-ol (diethylmethylcarbinol) and the unsaturated 3-methylpent-1-en-3-ol were also studied. Propargyl alcohol (prop-2-yn-1-ol) was also fed but proved to be lethal to rabbits in doses of

about 0·1 ml./kg. body wt. in about 24 hr., thus confirming observations made by Tietze (1926) that this compound was toxic.

Finally diethylaminoethanol, which is itself a compound of low pharmacological activity, but occurs as a constituent of many useful drugs, such as procaine, was included. Brodie, Lief & Poet (1948) injected 1 g. of diethylaminoethanol into human beings and found about 33 % to be excreted unchanged in the urine in 24 hr.; the fate of the rest is unknown.

EXPERIMENTAL

Materials and methods

Materials. The following commercial products were purified by distillation: 2-chloroethanol (ethylene chlorohydrin), b.p. 129°; trichloro-*tert.*-butanol (chlorbutol), m.p. 91°; 1:3-dichloropropan-2-ol (α:γ-dichlorohydrin), b.p. 174–175°; diethylaminoethanol, b.p. 159°/753 mm. Dichloroethanol, b.p. 146°; trichloroethanol, b.p. 55°/20 mm., and 2:2:3-trichloro-*n*-butanol, m.p. 60°, were prepared by the reduction of dichloroacetic acid, trichloroacetaldehyde (chloral) and 2:2:3-trichlorobutyraldehyde, respectively, with $LiAlH_2$ according to the general method described by Brown (1951). 1:1:1-Trichloropropan-2-ol (Isopral), b.p. 160°, n_D^{20} 1·4821, was prepared from chloral and magnesium methyl iodide (cf. Bayer and Co., 1904); it solidified on keeping.

3-Methylpentan-3-ol (diethylmethylcarbinol), b.p. 122°, n_D^{20} 1·418, was purchased. 3-Methylpent-1-yn-3-ol (ethylethynylmethylcarbinol; Oblivon), b.p. 119°, n_D^{20} 1·430, was the gift of British Schering Ltd. 3-Methylpent-1-en-3-ol (ethylmethylvinylcarbinol), b.p. 117–118°, n_D^{20} 1·425 (cf. Zal'manovich, 1948) was prepared by reducing 3-methylpentyn-3-ol in methanol with platinic oxide (Adams's catalyst) and hydrogen.

Methods. Glucuronic acid in urine was determined by the naphthoresorcinol method as modified by Paul (1951).

Rabbits (weighing about 3 kg.) were maintained on a constant diet of 50 g./day of rat cubes (diet 41, Associated London Flour Millers).

Most compounds were administered in aqueous suspension by stomach tube, at a dose of 1·5 mmole/kg. body wt. Monochloroethanol could not be administered at this level because of its toxicity. Propargyl alcohol (prop-2-yn-1-ol) was fed to three rabbits at a dose level of 0·1 ml./kg. body wt. but all three animals died within 12 hr.; the urine excreted was highly reducing to Benedict's reagent but no glucuronide was excreted (see Table 1 for results).

Isolation of glucuronides (glucosiduronic acids)

The glucosiduronic acids were isolated from the urine as basic lead salts (cf. Kamil *et al.* 1951), using the urine remaining from the quantitative analyses. In three cases, including trichloroethanol, the free glucosiduronic acids were isolated. In the other cases the glucuronide gums were converted into crystalline triacetyl methyl esters (cf. Kamil *et al.* 1951). These esters were obtained as colourless needles, insoluble in water but soluble in ethanol and $CHCl_3$. With the di- and tri-chloroethanols, the yield of crystalline ester was more than 1 g./g. of alcohol fed; with the other alcohols the yield varied from 50 to 300 mg./g. Optical rotations, melting points and elementary analyses for these compounds are given in Table 2.

The molecular rotations of the triacetyl methyl esters prepared here are quoted in Table 2. The $[M]_D$ of compounds containing the $-CCl_3$ group are more negative than the corresponding unsubstituted derivatives.

The following free acids were prepared; 1:1:1-*trichloro*-tert.-*butyl*-β-D-*glucosiduronic acid*, m.p. 170–171° and $[\alpha]_D^{20}$ – 37° in water (*c*, 1). (Found: C, 32·7; H, 4·5; Cl, 29·0. $C_{10}H_{15}O_7Cl_3$, H_2O requires C, 32·3; H, 4·6; Cl, 28·6 %); 2:2:3-*trichloro*-n-*butyl*-β-D-*glucosiduronic acid* (urobutylchloralic acid), m.p. 200–205° and $[\alpha]_D^{20}$ – 40° in water (*c*, 1). (Found: C, 31·6; H, 5·3. $C_{10}H_{15}O_7Cl_3$, 1·5 H_2O requires C, 31·6; H, 4·8 %.) The K and Ag salts of the latter acid, a metabolite of butyl chloral hydrate, have been described by Külz (1882) and Mering (1882).

Table 1. *Glucuronic acid conjugation of some aliphatic alcohols in rabbits*

Alcohol	Formula	Dose (mg./kg.)	Dose (mmole/kg.)	Conjugation (% of dose)	Conjugation Average (%)
Ethanol*	CH_3CH_2OH	770	5	—	0·5
Monochloroethanol	CH_2ClCH_2OH	80	1	(Toxic)	0?
Dichloroethanol	$CHCl_2CH_2OH$	170	1·5	34, 65, 67	55
Trichloroethanol	CCl_3CH_2OH	220	1·5	49, 52, 62	52
*iso*Propanol*	$(CH_3)_2CHOH$	1000	17	—	10
1:3-Dichloro*iso*propanol	$(CH_2Cl)_2CHOH$	190	1·5	21, 25, 37	28
(±)-1:1:1-Trichloro*iso*propanol	$(CH_3)(CCl_3)CHOH$	240	1·5	31, 37, 39	36
Butanol*	$CH_3(CH_2)_2CH_2OH$	370	5	—	2
(±)2:2:3-Trichloro-*n*-butanol	$CH_3CHClCCl_2CH_2OH$	260	1·5	39, 43, 60	47
tert.-Butanol*	$(CH_3)_3COH$	296	4	—	24
1:1:1-Trichloro-*tert.*-butanol	$CCl_3(CH_3)_2COH$	260	1·5	33, 43, 49	41
Diethylaminoethanol	$(C_2H_5)_2NCH_2CH_2OH$	170	1·5	0, 0, 0	0
3-Methylpentan-3-ol	$(C_2H_5)_2COHCH_3$	150	1·5	39, 44, 60	48
(±)-3-Methylpenten-3-ol	$(C_2H_5)(CH_2{:}CH)COHCH_3$	150	1·5	52, 60, 60	57
(±)-3-Methylpentyn-3-ol	$(C_2H_5)(CH{:}C)COHCH_3$	150	1·5	43, 53, —	48

* These alcohols are included for comparison and the values quoted are taken from Kamil *et al.* (1953*a*, *b*).

Table 2. *The properties of the triacetyl methyl esters of certain biosynthetic glucuronides*

	Triacetyl methyl esters							
				Elementary analysis				
		Optical rotation in $CHCl_3$ (*c*, 1)		Found		Formula requires		
β-Glucuronide	M.p.*	$[\alpha]_D^{20}$†	$[M]_D$	C	H	C	H	Formula
Ethyl‡	143°	−32·8°	−119°	—	—	—	—	—
2:2-Dichloroethyl	163–164	−23	−99	41·9	4·75	41·8	4·7	$C_{15}H_{20}O_{10}Cl_2$
2:2:2-Trichloroethyl	158	−37	−172	38·9	4·2	38·7	4·1	$C_{15}H_{19}O_{10}Cl_3$
*iso*Propyl‡	140	−37·8	−132	—	—	—	—	—
1:3-Dichloro*iso*propyl	145–146	−27	−118	43·7	4·9	43·2	5·2	$C_{16}H_{22}O_{10}Cl_2$
1:1:1-Trichloro*iso*propyl§	167–169	−33	−158	40·2	4·6	40·1	4·4	$C_{16}H_{21}O_{10}Cl_3$
2:2:3-Trichloro-*n*-butyl§	132–133	−19	−94	41·5	4·8	41·4	4·7	$C_{17}H_{23}O_{10}Cl_3$
tert.-Butyl‡	168	−29·2	−114	—	—	—	—	—
1:1:1-Trichloro-*tert.*-butyl	170	−28	−138	42·0	4·5	41·4	4·7	$C_{17}H_{23}O_{10}Cl_3$
3-Methylpentyl-3-ol	111	−24	−100	54·4	7·0	54·5	7·2	$C_{19}H_{30}O_{10}$
3-Methylpentenyl-3-ol§	99–105	−19	−79	55·0	6·9	54·8	6·8	$C_{19}H_{28}O_{10}$
3-Methylpentynyl-3-ol§	132–134	−14	−58	55·1	6·2	55·1	6·3	$C_{19}H_{26}O_{10}$

* Melting points are uncorrected.
† The error in $[\alpha]_D$ is ±1°.
‡ Included for comparison of rotations (values quoted from Kamil *et al.* 1953*a*, *b*).
§ The (±) alcohols were fed; the glucuronides are assumed to contain the aglycone in the (±) form.

DISCUSSION

The glucuronic acid conjugations of the alcohols studied are given in Table 1. It is to be noted that in all cases, except monochlorethanol, the introduction of chlorine atoms into the molecule causes a large increase in the glucuronic acid conjugation. Thus the oxidation of the alcohols appears to be inhibited by the presence of chlorine atoms. Whereas ethanol is largely oxidized to acetic acid, more than a half of the di- and trichloroethanols are excreted as glucuronides. However, no extra glucuronic acid excretion was detected in the urine during 12 hr. after dosing with monochloroethanol, and it seems probable that it is readily oxidized to monochloroacetic acid (the monochloroethanol was fed at a dose level of 80 mg./kg. body wt., but the animals died in 12 hr. after dosing; the LD_{50} for rats is 72 mg./kg. (Goldblatt, 1944; Goldblatt & Chiesman, 1944)). Monochloroacetic acid is a fairly toxic substance, being 25–40 times more toxic to rats, mice and guinea pigs than acetic acid and its di- and trichloro derivatives (Woodard, Lange, Nelson & Calvery, 1941). This acid also markedly depresses the respiration of mouse-liver slices under conditions where acetic acid and di- and tri-chloroacetic acids have little effect (Laug, 1946). It is reasonable to suppose that the toxicity of monochloroethanol is due to the production of monochloroacetic acid (cf. fluoroethanol and fluoroacetic acid; Reilly, Riker, Whitehouse & Kuriaki (1953) have suggested that the toxic action of monochloroacetate like that of monofluoroacetate may be due to an inhibition of pyruvate metabolism, although citrate accumulation does not occur with monochloroacetate). Thus it appears that glucuronic acid conjugation only becomes a significant metabolic path when two chlorine atoms have been substituted into the β-carbon of ethanol, and that di- and trichloroethanol are equally well conjugated. Butler (1948) has already shown that the oxidation of trichloroethanol to trichloroacetic acid in dogs is insignificant, the main metabolite being trichloroethyl glucosiduronic acid.

A similar effect is to be noted with 2:2:3-trichloro-*n*-butanol. Butanol itself is readily oxidized and has a low glucuronic acid conjugation in the rabbit (Kamil *et al.* 1953*a*), but in the 2:2:3-trichloro derivative two chlorine atoms occur on the β-carbon atom and this compound becomes highly conjugated with glucuronic acid.

The effect of chlorine substitution on the metabolism of a secondary alcohol is shown in the case of *iso*propanol. 1:2-Dichloro and 1:1:1-trichloro-*iso*propanol in low doses have much higher conjugations than *iso*propanol in high doses. Here again it appears that the oxidation to the ketone has been retarded. A similar effect is to be noted with *tert.*-butanol and its trichloro derivative.

Of the above chlorinated alcohols, trichloroethanol, 1:1:1-trichloro*iso*propanol (Isopral) and 1:1:1-trichloro-*tert.*-butanol (Chlorbutol) have found use as hypnotics, and it is possible that butyl chloral hydrate may be active because of reduction *in vivo* to 2:2:3-trichloro-*n*-butanol (cf. chloral hydrate). These four alcohols are excreted by rabbits highly conjugated with glucuronic acid.

It thus appears that chlorine substitution tends to retard the oxidation of alcohols and this may partly explain why the chlorinated alcohol has a

more powerful and more prolonged narcotic effect than the unsubstituted compound. However, conjugation with glucuronic acid is also an inactivating reaction, but it is localized mainly in the liver. It is also possible that chlorine substitution also retards the rate of glucuronic acid conjugation, so that the narcotic effectiveness of the substituted alcohol may be partly due to inhibition of oxidation and retardation of conjugation.

At the end of Table 1, values for the glucuronic acid conjugation of the three related tertiary alcohols, 3-methylpentan-3-ol, 3-methylpent-1-en-3-ol and 3-methylpent-1-yn-3-ol, are included to show the effect of unsaturation upon conjugation. The total conjugations of these three alcohols are not very different and it appears that the conversion of ethyl into vinyl or to ethynyl has little effect on conjugation. 3-Methylpentyn-3-ol (Oblivon, Dormison, Methylparafynol) has mild narcotic properties but nothing appears to be known about the narcotic properties of the other two alcohols.

SUMMARY

1. The glucuronic acid conjugation of a number of chlorinated and other aliphatic alcohols has been studied in the rabbit, and the glucosiduronic acids have been isolated as triacetyl methyl esters in each case.

2. Conjugation with glucuronic acid becomes a significant metabolic path when two or more chlorine atoms are present on the carbon atom next to that carrying the hydroxyl group. Thus 2-chloroethanol is not conjugated, whereas 2:2-di- and 2:2:2-tri-chloroethanols are highly conjugated (more than 50%).

3. It is suggested that chlorine substitution retards the oxidation of the alcohol and thereby increases its narcotic activity.

4. 3-Methylpentan-3-ol, 3-methylpent-1-en-3-ol and 3-methylpent-1-yn-3-ol are conjugated with glucuronic acid to about the same extent (50%). Diethylaminoethanol does not cause the excretion of extra glucuronic acid in rabbits.

We are grateful to British Schering Limited for a gift of methylpentyn-3-ol (Oblivon). The expenses of this work were in part defrayed by a grant from the Medical Research Council.

REFERENCES

Bayer & Co. (1904). *Chem. Zbl.* **1**, 1586; D.R.P. 151, 545.

Bock, H. (1930). *Inaug. Dissert. Breslau.* Cited by W. F. von Oettingen (1943). in *Publ. Hlth Bull., Wash.*, no. 281, p. 138.

Brodie, B. B., Lief, P. A. & Poet, R. (1948). *J. Pharmacol.* **94**, 359.

Brown, W. G. (1951). *Organic Reactions*, **6**, 469. New York: Wiley and Sons, Inc.

Burger, A. (1951). *Medicinal Chemistry*, **1**, 129. New York: Interscience.

Butler, T. C. (1948). *J. Pharmacol.* **92**, 49.

Butler, T. C. (1949). *J. Pharmacol.* **97**, 84.

Goldblatt, M. W. (1944). *Brit. J. industr. Med.* **1**, 213.

Goldblatt, M. W. & Chiesman, W. E. (1944). *Brit. J. industr. Med.* **1**, 207.

Kamil, I. A., Smith, J. N. & Williams, R. T. (1951). *Biochem. J.* **50**, 235.

Kamil, I. A., Smith, J. N. & Williams, R. T. (1953*a*). *Biochem. J.* **53**, 129.

Kamil, I. A., Smith, J. N. & Williams, R. T. (1953*b*). *Biochem. J.* **54**, 390.

Külz, E. (1882). *Pflüg. Arch. ges. Physiol.* **28**, 534.

Laug, E. P. (1946). *Proc. Soc. exp. Biol., N.Y.*, **61**, 178.

Lehmann, G. & Knoefel, P. K. (1938). *J. Pharmacol.* **63**, 453.

Lehmann, G. & Knoefel, P. K. (1939). *Amer. J. med. Sci.* **197**, 639.

Mering, E. von (1882). *Hoppe-Seyl. Z.* **6**, 492.

Paul, J. (1951). Ph.D. Thesis, University of Glasgow.

Reilly, J., Riker, W. F., Whitehouse, W. C. & Kuriaki, K. (1953). *J. Pharmacol.* **108**, 393.

Tietze, K. (1926). *Med. Klinik*, **22**, 1843.

Woodard, G., Lange, S. W., Nelson, K. W. & Calvery, H. O. (1941). *J. industr. Hyg.* **23**, 78.

Zal'manovich, M. Z. (1948). *J. gen. Chem., Moscow*, **18**, 2103. Cited in *Chem. Abstr.* (1949), **43**, 3777.

A Note on the Estimation of Sphingomyelin in Nervous Tissue

By R. M. C. DAWSON*
Department of Biochemistry, University of Oxford

(*Received* 13 *November* 1953)

The estimation of sphingomyelin in animal tissues is almost exclusively carried out by measuring the lipid phosphorus which is stable to mild alkaline and acid hydrolysis. Apart from sphingomyelin, all the known phospholipids are decomposed by incubating with N potassium hydroxide at 37° followed by standing at room temperature at an acid pH (Schmidt, Benotti, Hershman & Thannhauser, 1946). However, the work of Brante (1949) has suggested that certain kephalin fractions isolated from cerebral tissue contain some lipid phosphorus which is stable to mild alkaline and acid hydrolysis, although the fractions themselves contain no choline.

* Betty Brookes Research Fellow.