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A RAPID, SIMPLE, AND MILD PROCEDURE FOR ALKYLATION OF PHENOLS, ALCOHOLS, AMIDES AND ACIDS

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Abstract—A general, rapid method is described for alkylation of phenols and alcohols to give ethers, for amides to give N-substituted amides, and for acids to give esters. Differences in optimum reaction times suggest that where two or more such groups as phenol, alcohol, amide, and acid occur in the same molecule, differential alkylation could be effected with suitable substrates. Alkylation with primary alkyl halides was very effective but secondary halides showed evidence for competitive dehydrohalogenation before alkylation was complete and tertiary halides were rapidly dehydrohalogenated with no formation of alkylated derivatives. The method has been applied successfully to N,O-alkylation of peptides for mass spectrometric sequence determination. C-Methylation of peptidic amino-acid residues was observed only on carbon α to a carboxylic ester.

In connection with other work,[1-3] a mild, rapid methylation of secondary amides was required. Methods for effecting methylation already exist[4] but none is ideal, requiring strong bases and dry conditions. For the special case of trifluoroacetamides, which have a very acidic amide hydrogen, methylation (or alkylation generally) can be effected quickly and easily using solid KOH in acetone;[3] subsequent rapid hydrolysis affords the amine. Recently, attempts have been made to improve this process, particularly for alkylation with alkyl chains of over three C atoms.[5] In the latest work, the appropriate alkyl halide is reacted in the presence of very strong base and the overall yields for the reactions involving longer alkyl chains are not especially good despite the very long reaction times employed. Together with our work using solid KOH as base with trifluoroacetamides,[3] a reported N-alkylation of indoles and pyrroles using KOH in dimethyl sulphoxide (DMSO)[6] suggested that solid KOH might be used for alkylation of any amide. Not only was this supposition found to be correct in the work described here but it was found also that phenols, alcohols, and acids were alkylated conveniently by a similar procedure. Thus, after greatly simplyifying the method for N-alkylation of indoles and pyrroles,[6] this reaction has been extended to include N- and/or O-alkylation of other groups having acidic hydrogens. Typical results are shown in Table 1.

DISCUSSION

In the process described here, the substrate and alkyl halide were simply added to powdered KOH stirred in DMSO, usually at room temperature unless stated otherwise (Table 1). Little KOH actually dissolves, its solubility in DMSO being low.[7] As an additional advantage, it was found to be unnecessary to use especially dry DMSO nor to take special precautions against ingress of atmospheric moisture.

For alcohols, phenols, amides, and acids, methylation occurred readily. Introduction of larger alkyl groups was slower, particularly for the sterically hindered cases especially chosen for Table 1 but reaction was still clean and gave only unreacted starting material besides the desired product after the stated times. No extensive series of experiments were carried out to improve the quoted yields. For example, 1-bromobutane gave a 99% yield of N-1-butylacetanilide after 30 minutes at room temperature but the more sterically crowded triphenylmethanol afforded only 45% of the O-1-butyl ether after 60 min at 55°. In contrast, for both substrates, methylation proceeded in over 90% yield at room temperature with times of 5 and 30 min respectively. Methylation was generally almost complete within 10 min but frequently a 30 min reaction time was used to guarantee the highest yields. The higher temperature and longer time required for di-methylation of 1,2-di-(1-hydroxycyclohexyl)-acetylene were necessary because of its limited solubility in DMSO.

Alkylation of acids was always slower requiring, for example, 60 min at room temperature to achieve an 86% yield of methyl hexanoate from hexanoic acid. Formation of esters under these alkaline conditions is remarkable but suggests, along with other later evidence, that reaction occurs on the surface of the KOH and may be in a similar category to those in which solid hydrogen carbonate or potassium carbonate[8] or silver oxide[9] are used as base in non-aqueous solvents to convert acids to esters. We did not find significant alkylation to occur with potassium carbonate used in place of KOH in the reactions described here. For secondary alkyl halides, it has been suggested that dehydrohalogenation is competitive with N-alkylation for indoles and pyrroles with KOH/DMSO.[6] For tertiary halides, dehydrohalogenation is so fast that alkylation is excluded entirely. These reports are confirmed in our work

Table 1. Alkylation with KOH/DMSO

Substrate	Alkyl halide	Product(s)	Yield (%)	Reaction Conditions[a]
$C_6H_5CONH_2$	CH_3I	$C_6H_5CON(CH_3)_2$	99	5
$C_6H_5NHCOCH_3$	CH_3I	$C_6H_5N(CH_3)COCH_3$	91[b]	5
	C_2H_5Br	$C_6H_5N(C_2H_5)COCH_3$	95	30
	n-C_3H_7Br	C_6H_5N(n-C_3H_7)$COCH_3$	94	30
	n-C_4H_9Br	C_6H_5N(n-C_4H_9)$COCH_3$	99	30
	$(CH_3)_2CHBr$	$C_6H_5NHCOCH_3$	65[c]	30
		$C_6H_5N[CH(CH_3)_2]COCH_3$	25[c]	
	$(CH_3)_2CHBr$	$C_6H_5NHCOCH_3$	29[c]	60(55[d])
		$C_6H_5N[CH(CH_3)_2]COCH_3$	69[c]	
	$(CH_3)_3CBr$	$C_6H_5NHCOCH_3$	93	30
	$(CH_3)_3CBr$	$C_6H_5NHCOCH_3$	83	60(55[d])
C_6H_5OH	CH_3I	$C_6H_5OCH_3$	77	5
			85	10
			83	30
$C_8H_{17}OH$	CH_3I	$C_8H_{17}OCH_3$	85	10
			99	30
2-$HOC_6H_4CH_2OH$	CH_3I	2-$CH_3OC_6H_4CH_2OCH_3$	97	30
$[CH_2(CH_2)_4C(OH)—CH_3]_2$	CH_3I	$[CH_2(CH_2)_4C(OCH_3)—CH_3]_2$	89	60(55[d])
$(C_6H_5)_2CHOH$	CH_3I	$(C_6H_5)_2CHOCH_3$	97	30
$(C_6H_5)_3COH$	CH_3I	$(C_6H_5)_3COCH_3$	96[b]	30
	C_2H_5Br	$(C_6H_5)_3COC_2H_5$	73[b]	30
	n-C_3H_7Br	$(C_6H_5)_3COH$	62[c]	30
		$(C_6H_5)_3COC_3H_7$(n)	38[c]	
	n-C_3H_7Br	$(C_6H_5)_3COH$	57[c]	60(55[d])
		$(C_6H_5)_3COC_3H_7$(n)	43[c]	
	n-C_4H_9Br	$(C_6H_5)_3COH$	50[c]	60(55[d])
		$(C_6H_5)_3COC_4H_9$(n)	45[c]	
$C_5H_{11}CO_2H$	CH_3I	$C_5H_{11}CO_2CH_3$	44	5
			80	30
			85	60
$(CH_3)_3CCONH_2$	CH_3I	$(CH_3)_3CCONHCH_3$	52[b,d]	5

[a] Reaction time given in minutes. Reaction temperature is ambient (ca 18–20°) unless stated otherwise in parenthesis.
[b] Yield after recrystallisation.
[c] Yield calculated from ^1H NMR spectrum of mixture and based on total recovery of materials. No signals other than those expected for the two substances were observed, except for traces of solvent.
[d] See also Table 2.

Table 2. Results of methylation of trimethylacetamide

Method[a]	Reaction time (min)[b]	Product ratio[c]
KOH/DMSO/CH_3I	1	32:65: 3
	2	18:78: 4
	5	14:71:15
	10	9:74:17
	15	9:77:14
	30	14:75:11
	60	5:75:20
	120	10:70:20
	180	10:72:18
Dimsyl sodium/ DMSO/CH_3I	15	3:54:43

[a] See experimental for details.
[b] All reactions were done at ambient temperature (ca 18–20°).
[c] Product ratio for respectively, $(CH_3)_3CCONH_2$:$(CH_3)_3CCONHCH_3$:$(CH_3)_3CCON(CH_3)_2$

(Table 1). Thus, at room temperature, 2-bromopropane yielded only 25% of N-2-propylacetanilide after 30 minutes whereas, under the same conditions, 1-bromopropane gave a 94% yield of N-1-propylacetanilide. It is unlikely that these results reflect a true comparison of rates of alkylation and dehydrohalogenation because of the quite severe steric effects militating against N-alkylation with a secondary alkyl group. In fact, it is notable that simply increasing the time and temperature (Table 1) increased the yield of N-2-propylacetanilide from 25 to 69%. Steric effects were marked also in the N-methylation of trimethylacetamide (pivalamide) which, unlike other less sterically-crowded primary amides gave a 52% yield of mono-N-methylated product, with some di-N-methylated material indicated by NMR spectroscopy of the crude product. This result may be compared with the 99% yield of di-N-methylated benzamide in the same time (Table 1) and the larger yields of mono- and di-N-methylated trimethylacetamide using the stronger base, dimsyl sodium, for 15 min (Table 2). Therefore, although alkylation with secondary alkyl halides is slower than with primary halides, it seems that much of the reduced rate may be due to steric effects and therefore that lower yields for comparable times for primary and secondary halides are not due to excessively competitive dehydrohalogenation. Use of excess of halide and extended times removes this difficulty. Alkylation with tertiary halides must be so slow and dehydrohalogenation so fast in KOH/DMSO that N- or O-alkylation is impossible. Vigorous evolution of gas was observed on addition of tertiary halides to KOH and DMSO.

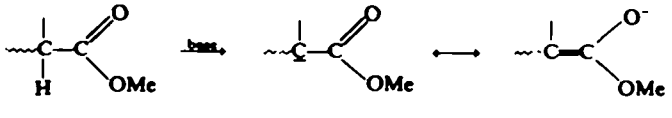
This convenient method for preparation of ethers, substituted amides, and esters has two limitations.[1] First, if an aliphatic amine is present, a quaternary salt is likely to be formed. For example, methylation of 1,2,3,4-tetrahydroisoquinoline using the original procedure[6] gave no ether-soluble product. When the amount of iodomethane was reduced, a small yield (24%) of tertiary amine could be isolated. Secondly, when a highly activated methylene group is present, C-methylation is likely. Thus, phenylacetamide gave mainly N,N-dimethylphenylacetamide plus some N,N-dimethyl-2-phenylpropanamide.

Application to peptides. Because N-methylation of secondary amides proceeded rapidly and cleanly under mild conditions, the method was investigated further as a means of per-N,O-methylation of peptides to aid mass spectrometric sequencing of amino-acids. The first experiments in this area used silver oxide[10] as the base and, more recently, sodium hydride[11] or dimsyl sodium (Hakomori method)[12,13] have been used extensively. The last method used the powerful base, dimsyl sodium, in DMSO and C-methylation is frequently observed;[14–16] for this reason, and to avoid quaternisation of amines and sulphides, times are as short as one minute with the danger of undermethylation. C-Methylation is particularly evident with glycyl residues but the use of trideuterioiodomethane allows this side-reaction to be distinguished in the mass spectrum. Accordingly, actual C-methylation is not a drawback but incomplete C-methylation is, leading as it does to mixtures of peptides and 'mixed' mass spectra.

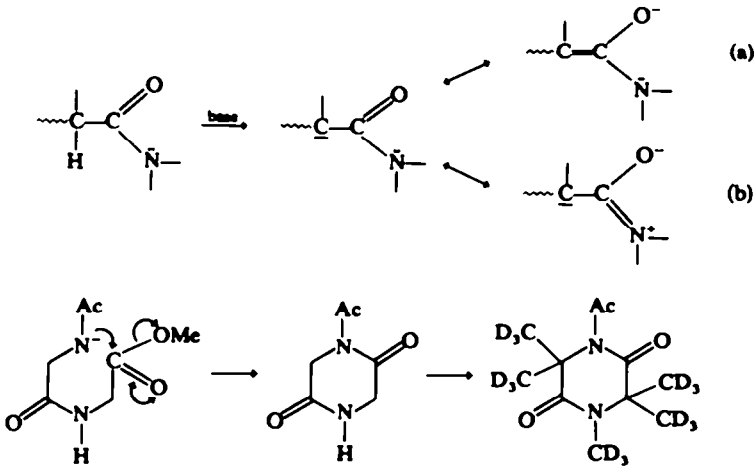
The applicability of the KOH/DMSO method for permethylation of peptides was tested on a large-scale with methyl N-acetylglycinate and dimethyl N-acetylglutamate. After reaction times of 30 minutes and 10 min respectively at room temperature, the yields of crude, isolated N-methylated products were 57 and 35%. However, in each case, NMR spectroscopic analysis showed that each product was virtually pure with no evidence for unchanged starting material. The relatively low yields are ascribed to poor recovery, the products being soluble in aqueous solvents. Methyl-N-acetylglycinate was not C-methylated whereas the same compound and its parent acid with dimsyl sodium in DMSO for 2 min, afforded a large proportion of the N- and C-methylated product.

As esterification is rather slow with KOH/DMSO, amino-acids or peptides were N-methylated after prior conversion to their methyl esters. The latter enhance the possibility of C-methylation particularly with stronger bases like dimsyl sodium or sodium hydride. Acidities of amide hydrogens and hydrogens α to an ester group are very similar.[17] To reproduce conditions likely to be encountered in sequencing unknown peptides, a very large excess of base (KOH) and iodomethane (or trideuterioiodomethane) were reacted with peptides, acetylated at the N-terminus and esterified at the C-terminus, at the 50–100 nmol level. Under these conditions, specific C-methylation was observed on the α-carbon adjacent to any ester group. This C-methylation was not observed in the larger-scale experiments described above using a much smaller excess of base and iodomethane. Presumably, a negative charge developing on the carbon α to an ester group is resonance stabilized (i) whereas a carbon α to an amide group is not so stabilized (iia) because of the stronger cross-conjugative effect of the amide itself (iib) due to the greater electronegativity of O compared with N. Thus, in any peptide, when using a mild base, only C-terminal residues are likely to be C-methylated along with any side-chain carrying an ester function (Glu, Asp). These C-methylations can be distinguished by using trideuterioiodomethane and by observation of A- and B-type ions[14] in the mass spectrum. The following examples have been chosen to illustrate these points.

Permethylation of Ac.Gly.Ala.Phe.Ala.Gly.OMe with KOH/DMSO/MeI gave a product, the mass spectrum of which was readily interpreted (**1**) with all A- and B-type sequence ions present. The N-terminal glycyl residue was not C-methylated at all



(i)



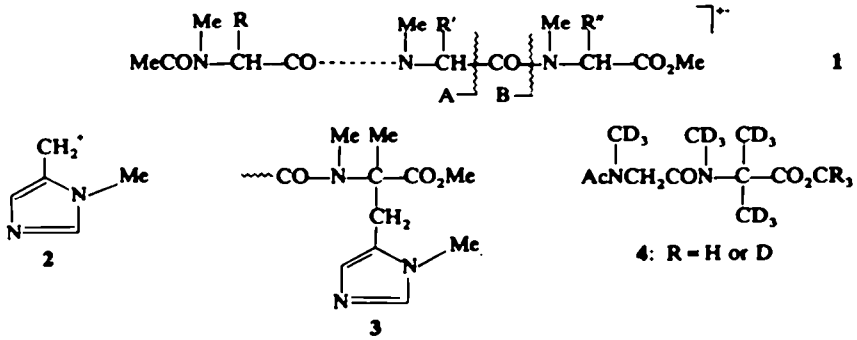
but the C-terminal glycyl was fully C-methylated and the peptide gave a molecular ion 28 amu more than that expected without C-methylation. Similarly, Ac.Gly.Pro.Trp.Leu.OMe was per-N-methylated and mono-C-methylated at leucine; the N-terminal glycyl residue was not C-methylated. For the dipeptide, Ac.Gln.His.OMe, two products were observed. The more volatile showed the sequence ions expected for per-N-methylation, including an ion at m/z 95 (2), characteristic of the histidyl side-chain. The second product to volatilize in the mass spectrometer showed the histidyl residue had been C-methylated but, since the ion at m/z 95 was still present, the C-methylation must have occurred adjacent to the ester group (3). There was no evidence for extensive quaternization of the histidyl side-chain nitrogens.

In the tetrapeptide, Ac.Ala.Glu.(OMe).Leu.Gly.OMe, complete C-methylation of the terminal glycyl residue occurred and mono-C-methylation of the side-chain of the glutamyl ester α to the ester group.

Curiously, for the very simple dipeptide, Ac.Gly.Gly.OMe, reaction with CD_3I/KOH/DMSO gave the fully deuteriomethylated diketopiperazine (iii) whilst, with CD_3I and dimsyl sodium in DMSO, both it and Ac.Gly.Gly.OH gave an uncyclised N- and C-methylated product (4). Perhaps for this readily cyclised dipeptide, the negative charge developing on the terminal amide nitrogen as KOH begins to remove a proton can attack the C-terminal ester function before the proton is fully removed (iii). With the stronger base, dimsyl sodium, removal of the proton may well be faster, allowing normal nucleophilic attack on iodomethane. Like the esterification of acids observed with KOH, these results also suggest that reaction proceeds on the surface of the solid KOH so that the very small quantity of KOH actually dissolved in the DMSO does not function effectively in its normal solution mode. All of the reactions in which solid KOH in DMSO has been used in these experiments have involved initial removal of a proton from the substrate and hydrolysis is not observed.

CONCLUSION

The N- and/or O-alkylation of alcohols, phenols, amides, and acids described here is rapid and simple for primary and secondary alkyl halides but does not work with tertiary halides. For permethylation of peptides, it is an easy practical technique yielding good, clear mass spectra. No special precautions are necessary to keep the reagents dry. C-Methylation of all amino-acid residues α to an ester group was observed consistently, and can be monitored readily through use of trideuteriomethane. It would be invidious to draw extensive conclusions from a comparison of this method and Hakomori's for permethylation of peptides on the basis of a relatively small number of compounds and in the face of the widespread popularity of the latter but the present method gives good recovery of material and the position of C-methylation appears to be predictable, unlike the



reported experiences of others[14-16] using the Hakomori method. Any OH groups in amino-acid side-chains are also methylated consistently by the present technique.

EXPERIMENTAL

M.ps are uncorrected. PMR spectra were recorded on a Perkin–Elmer R12B spectrometer. Mass spectra were obtained from an AEI MS 12 spectrometer at 70 eV. All chemicals were either commercial reagent grade or were synthesised by standard methods. Dimethyl sulphoxide was stored over molecular sieve (type 4A) but, in experiments, no other precautions were taken against ingress of moisture.

General alkylation procedure (for non-peptidic substrates)

(a) To DMSO (2 ml) was added powdered KOH (4 mmol per replaceable hydrogen of substrate). After stirring for 5 min, the substrate (1 mmol) was added, followed immediately by the alkyl halide (2 mmol per replaceable hydrogen of substrate). Stirring was continued for the stated time (Table 1 and 2) after which the mixture was poured into water (20 ml) and extracted with dichloromethane (3 × 20 ml). The combined organic extracts were washed with water (5 × 10 ml) and filtered through cotton wool to remove water. Rotary evaporation of the filtrate gave the product for which NMR spectra and, where appropriate (Tables 1, 2), melting points were obtained.

(b) Methylation with dimsyl sodium/DMSO/MeI was performed by a standard method[18] with a 2 min reaction time. The reaction products were worked up as in (a).

General procedure for peptides (small scale)

(a) The peptide (50–100 nmol) was dissolved in Ac_2O/MeOH (40 μl, 1:3) and shaken vigorously. The soln was left to stand at room temp for 3 hr. The N-acetyl peptide was isolated by evaporation *in vacuo*. To the residue dissolved in MeOH (250 μl) was added an ethereal soln of diazomethane until the soln remained yellow on standing at room temp for about 30 min. AcOH was added to destroy excess of diazomethane and the acetylpeptide methyl ester was isolated by evaporation *in vacuo*. To this residue, dissolved in DMSO (100–200 μl), was added powdered KOH (11–22 mg; 0.2–0.4 mmol) followed immediately by iodomethane or trideuterioiodomethane (6–12 μl; 0.1–0.2 mmol). The mixture was shaken vigorously for 10 min and then quenched by the addition of water (0.6 ml). The product was extracted into chloroform (3 × 200 μl) and the combined organic extracts were washed with water (3 × 200 μl). Evaporation in a vacuum desiccator gave the solid product which was dissolved in MeOH (20 μl). Half of the resulting soln was used for mass spectrometric analysis.

(b) Methylation of the peptide derivative (50–100 nmol) with dimsyl sodium/DMSO/CH_3I or CD_3I was carried out as described[19] with a reaction time of 90 sec. Work-up was as in (a).

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REFERENCES

[1]M. E. Rose, D. Phil. Thesis. University of York (1978).
[2]R. A. W. Johnstone and T. J. Povall, *J. Chem. Soc.* Perkin I, 1297 (1975).
[3]R. A. W. Johnstone, D. W. Payling, and C. Thomas, *Ibid.* (C), 2223 (1969).
[4]See, for example, W. S. Fones, *J. Org. Chem.* **14**, 1099 (1949). J. B. Hendrickson and R. Bergeron, *Tetrahedron Letters* 345 (1970); M. G. Ahmed, R. W. Alder, G. H. James, M. L. Sinnott, and M. C. Whiting, *J. Chem. Soc.* Chem. Comm. 1533 (1968); H. Meerwein, *Methoden der Organischen Chemie*, Part 3, Vol. 6, p. 325; Georg Thieme Verlag, Stuttgart (1965), and refs 3, 5, 10–13.
[5]J. E. Nordlander, D. B. Catalane, T. H. Eberlein, L. V. Farkas, R. S. Howe, R. M. Stevens, N. A. Tripoulas, R. E. Stransfield, J. L. Cox, M. J. Payne, and A. Viehbeck, *Tetrahedron Letters* 4987 (1978)
[6]H. Heaney and S. V. Ley, *J. Chem. Soc.* Perkin I, 499 (1973).
[7]D. Martin and H. G. Hanthall, *Dimethyl sulphoxide* p. 132. Van Nostrand, Wokingham (1975).
[8]For some examples of this old procedure see, T. H. Simpson and J. L. Beton, *J. Chem. Soc.* 4065 (1954).
[9]C. Liebermann and S. Lindenbaum, *Ber. Dtsch. Chem. Ges.* **42**, 1397 (1909).
[10a]B. C. Das, S. D. Gero, and E. Lederer, *Biochem. Biophys. Res. Comm.* **29**, 211 (1967); [b]K. L. Agarwal, R. A. W. Johnstone, G. W. Kenner, D. S. Millington, and R. C. Sheppard, *Nature* **219**, 498 (1969).
[11]G. Marino, L. Valente, R. A. W. Johnstone, F. Mohamedi-Tabrizi, and G. C. Sodini, *J. Chem. Soc.* Chem. Comm. 357 (1972).
[12a]S. I. Hakomori, *J. Biochem. Tokyo* **55**, 205 (1964); [b]D. W. Thomas, *Biochem. Biophys. Res. Comm.* **33**, 483 (1968).
[13a]H. R. Morris, *FEBS Letters* **22**, 257 (1972); [b]H. R. Morris, R. J. Dickinson, and D. H. Williams, *Biochem. Biophys. Res. Comm.* **51**, 247 (1973).
[14]P. J. Arpino and F. W. McLafferty, *Determination of Organic Structures by Physical Methods* (Edited by F. C. Nachod, J. J. Zuckermann, and E. W. Randal) Vol. 6, p. 1, Academic Press, New York (1976).
[15]R. Hodges, S. B. H. Kent, and B. C. Richardson, *Biochim. Biophys. Acta* **257**, 54 (1972).
[16]M. Mudgett, D. V. Bowen, T. J. Kindt, and F. H. Field, *Biomed. Mass Spectrom.* **2**, 254 (1975).
[17]J. March, *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure* p. 221 McGraw-Hill, Tokyo (1968).
[18]M. L. Polan, W. J. McMurray, S. R. Lipsky, and S. Lande, *Biochem. Biophys. Res. Comm.* **38**, 1127 (1970).
[19]H. R. Morris, D. H. Williams, and R. P. Ambler, *Biochem. J.* **125**, 189 (1971).