The Formation of 1-Dimethylaminonaphthalene-5-sulphonamide during the Preparation of 1-Dimethylaminonaphthalene-5-sulphonyl-amino Acids

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1. The amount of 1-dimethylaminonaphthalene-5-sulphonamide formed during the reaction of an amino acid with 1-dimethylaminonaphthalene-5-sulphonyl chloride depends on the structure of the amino acid and on the conditions used. 2. The reaction probably involves attack of a further molecule of 1-dimethylaminonaphthalene-5-sulphonyl chloride on the 1-dimethylaminonaphthalene-5-sulphonyl-amino acid and also gives the aldehyde (or ketone) with one carbon atom less than the parent amino acid.

DNS chloride* reacts with amino acids in buffered aqueous acetone solution to give the highly fluorescent DNS-amino acids (Hartley & Massey, 1956). The reagent has been used by Gray & Hartley (1963*a*,*b*) in the identification of *N*-terminal residues of peptides on a millimicromolar scale. Methods have also been reported for the separation of complex mixtures of DNS-amino acids by paper (Boulton & Bush, 1964) and thin-layer (Seiler & Wiechmann, 1964) chromatography. DNS-NH_2 is produced as an artifact under the conditions normally used for labelling amino acids. The present paper describes experiments aimed at determining the origin of this material and the mechanism of its formation.

MATERIALS AND METHODS

Materials. The DNS chloride used was obtained from British Drug Houses Ltd., Poole, Dorset, and the other materials were from the usual commercial sources.

Determination of 1-dimethylaminonaphthalene-5-sulphonamide. Reactions were carried out at room temperature (about 20°) in subdued light in stoppered tubes. Portions of the reaction mixtures were applied directly to thin layers of silica gel G (E. Merck A.-G., Darmstadt, Germany) supported on glass plates. Chromatography was carried out in the systems described by Seiler & Wiechmann (1964), and the zones containing fluorescent material were marked. In each case the adsorbent was removed, and the product was eluted from it with methanol and determined fluorimetrically with the Locarte Mk. 4 fluorimeter. The light source was a mercury arc lamp. A primary filter transmitting between 340 and 380 mμ and a secondary filter transmitting only above 510 mμ were used. In calculating the results it was assumed that the molar fluorescence yields were the same for the different compounds.

Characterization of the aldehyde. L-Leucine (1 m-mole), DNS chloride (3·7 m-moles) and $NaHCO_3$ (12 m-moles) were allowed to react at room temperature for 100 min. in a mixture of dioxan (30 ml.) and water (20 ml.). A portion of the reaction mixture was then distilled into Brady's (1931) reagent, and the 2,4-dinitrophenylhydrazone of isovaleraldehyde (0·51 m-mole) was isolated by conventional methods. The identity of this with an authentic sample was confirmed by m.p., by infrared spectrum and by thin-layer chromatography on alumina G (E. Merck A.-G.) in

* Abbreviations: DNS, 1-dimethylaminonaphthalene-5-sulphonyl; DNS-NH_2, 1-dimethylaminonaphthalene-5-sulphonamide.

Table 1. *Proportions of 1-dimethylaminonaphthalene-5-sulphonamide to 1-dimethylaminonaphthalene-5-sulphonylamino acid formed with different amino acids*

The reaction mixture consisted of 0·1 m-mole of the amino acid, 0·11 m-mole of DNS chloride and 0·5 m-mole of $KHCO_3$ in acetone (4·5 ml.) and water (3·5 ml.). The reactions were allowed to go substantially to completion at room temperature in subdued light. The value given is the amount of amide as a percentage of the total (DNS-NH_2 + DNS-amino acid).

Amino acid	DNS-NH_2 (% of total)	Amino acid	DNS-NH_2 (% of total)
None	< 1	α-Alanine	11
Glycine	2	Valine	12
Sarcosine	< 1	Leucine	15
Proline	< 1	Norleucine	15
β-Alanine	1·5	α-Aminoisobutyric acid	45

Scheme A:

$$\text{DNS-NH}\cdot\text{CHR}\cdot\text{CO}_2^- + \text{DNS-Cl} \longrightarrow \text{DNS-NH}\cdot\text{CHR}\cdot\text{CO}\cdot\text{O-DNS (I)} + \text{Cl}^-$$

$$\text{(I)} \xrightarrow{\text{OH}^-} \text{DNS-NH}_2 + \text{R}\cdot\text{CHO} + \text{CO} + \text{DNS-O}^-$$

Scheme B:

$$\text{Ts-NH}\cdot\text{CHR}\cdot\text{CO}\cdot\text{Cl} \xrightarrow{\text{OH}^-} \text{Ts-NH}_2 + \text{R}\cdot\text{CHO} + \text{CO} + \text{Cl}^-$$

Ts = toluene-*p*-sulphonyl

the systems diethyl ether–light petroleum (b.p. 40–60°) (5:14, v/v), ethyl acetate–hexane (1:9, v/v) and benzene–light petroleum (b.p. 40–60°) (3:1, v/v) (Nano, 1964).

RESULTS

The relative proportions of DNS-NH_2 and DNS-amino acid formed under comparable conditions with different amino acids are shown in Table 1. In most cases only a small part of the amide formed in the labelling of the amino acid can be accounted for by contaminants in the reagents (cf. Gray & Hartley, 1963*a*,*b*, for peptides). The remainder appears to have come from the amino acid itself, the proportion formed depending on the structure of the amino acid. In particular, sarcosine, proline, β-alanine and glycine give little or no DNS-NH_2 (or DNS-NHMe). It was noted during this experiment that the reaction of α-aminoisobutyric acid with DNS chloride is markedly slow. This is undoubtedly due to steric hindrance around the amino group and would be expected to affect the proportion of DNS-NH_2 formed, since the relatively small amount of DNS-α-aminoisobutyrate formed will be exposed to a correspondingly greater amount of unchanged DNS chloride (see below).

Increasing the proportion of DNS chloride in the reaction mixture gives increased amide formation. Thus, with 2·2m-moles of DNS chloride under the conditions indicated in Table 1, α-alanine gives 39% of DNS-NH_2. This suggests that the formation of the amide involves 2mol. of DNS chloride/mol. Confirmation of this is obtained by treating a solution of DNS-leucine [purified by chromatography on a dry cellulose column with butanol–acetic acid–water (12:3:5, by vol.)] with DNS chloride and bicarbonate in aqueous acetone, when DNS-NH_2 is formed in considerable quantity.

The decomposition of the DNS-amino acid gives, in addition to the DNS-NH_2, the aldehyde (or ketone) with one less carbon atom (C-1) than the parent amino acid. Thus, from the reaction of leucine with an excess of DNS chloride in aqueous dioxan, isovaleraldehyde (3-methylbutyraldehyde) can be isolated as its 2,4-dinitrophenylhydrazone. Similarly, valine gives the 2,4-dinitrophenylhydrazone of isobutyraldehyde, and α-aminoisobutyric acid the 2,4-dinitrophenylhydrazone of acetone. The pungent smell of isovaleraldehyde could also be detected from the reaction mixture of DNS-leucine itself with DNS chloride.

DISCUSSION

These results are consistent with the reaction scheme A. The mode of decomposition of the mixed anhydride (I) is proposed by analogy with the decomposition of toluene-*p*-sulphonylamino acid chlorides (scheme B) (Wiley & Davis, 1954; Beecham, 1957). The structural requirements for the latter reaction are: (*a*) that the amide and –CO·Cl groups are joined to the same carbon atom (C-2 of the amino acid); (*b*) that there is a free hydrogen atom on the amido nitrogen; (*c*) that an electron-donating alkyl group (R) is present on C-2. These requirements seem to be paralleled by the requirements for DNS-NH_2 formation in the reaction of the amino acid with DNS chloride. We have not, however, attempted to demonstrate the presence of carbon monoxide in the reaction mixture so that the exact analogy has not been rigidly established.

These findings have obvious relevance to the problem of determination of amino acids by the DNS method (Boulton & Bush, 1964). Complete conversion of the amino acid demands that a significant excess of DNS chloride be used, but on the other hand the larger the excess the greater the proportion of amide formed. A consideration of the kinetics of these reactions also suggests that the overall dilution of the solution should affect the ratio of DNS-NH_2 to DNS-amino acid formed.

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