BROMATOMETRIC DETERMINATION OF PHENOLS AND PHENOLIC CARBONYL COMPOUNDS

KOPPESCHAAR'S[1] bromatometric method is extensively used for the determination of phenols and related compounds. In practice two procedures are used which differ significantly in the magnitude of the excess of the brominating mixture added. The complications which may occur in this method are discussed elsewhere[3–9].

Neelakantam and Viswanath[10] investigated the bromination of the phenolic ketone and resacetophenone, by the excess method and found that it did not stop at either the the oretical dibromination or even the tribromination stage. Direct titration with the brominating mixture using methyl orange as indicator was employed by Ramanujam[11] for the determination of the ketone and also for the analysis of the copper complex[12], and by Raja Reddy for phenol[13]. The results corresponded to di- and monobromination respectively for the ketone and its monomethyl ether. This method appears to be better suited for the volumetric analysis of metallic complexes with *o*-hydroxy carbonyl compounds.

In the present investigation the determination of phenols and phenolic carbonyl compounds was carried out following the procedure described by Raja Reddy[13] at 30–35° C.

The results are summarised below:

1. Phenol[3,6], undergoes tribromination.
2. Salicylic acid undergoes dibromination without decarboxylation. In the excess method, it undergoes tribromination and decarboxylation above 20° C[2,3,6].
3. *p*-Cresotic acid (6-hydroxy-3-methyl benzoic acid) undergoes dibromination and decarboxylation.
4. Thymol[6] (6-isopropyl-*m*-cresol) undergoes dibromination.
5. Vanillin[3] undergoes dibromination with the elimiantion of the aldehyde group whereas it undergoes monobromination at 0° C.
6. Pyrogallol undergoes dibromination and gallic acid undergoes monobromination only.
7. *p*-Hydroxyacetophenone undergoes dibromination.
8. β-Naphthol[6] undergoes monobromination and 2-hydroxy-3-naphthoic acid, dibromination and decarboxylation.

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SOME OBSERVATIONS ON REGULAR STRUCTURES IN PROTEINS

EVER SINCE Pauling discovered α-helix[1], it was expected that the globular protein molecules would contain high percentage of α-helices because of the great stability of this conformation. But when crystal structures of many proteins were solved, it was apparent that it was not quite so. Many of the globular proteins were found to contain β-sheets too, which are the other predicted low-energy conformation of polypeptide chains[2]. Apart from these regular structures of α-helices and β-sheets, considerable portion of the polypeptide chains in globular proteins contain nonregular conformations, where the successive units do not have repeating conformational features.

The purpose of this note is to attract attention to an interesting feature of protein folding. In almost all the proteins roughly half the number of the residues are involved in either of the two regular structures, *viz.*, α-helices and β-sheets. Figure 1 shows the plot of a number of residues involved in these two regular conformations, against the total number of residues, for various protein molecules (the data have been compiled from refs. 3–5). The abbreviations and the corresponding common names of the proteins shown in this figure are; INS—Insulin; PTI—Pancreatic trypsin inhibitor; CYT—Cytochrome; MGN—myoglobin; CPM—carp muscle calcium binding protein; LYZ—Lysozyme; TPG—Trypsinogen; CNA—Concanavalin A; LST—Elastase; SBT—

Subtilisin; CPA—Carboxypeptidase A: LDH—Lactate dehydrogenase.

It is seen from Fig. 1 that about 45% of the total number of residues in a protein are having regular conformations of α-helices or β-strands. A straight line corresponding to this value of 45% has been drawn in the figure to show how closely the rule is followed. A tentative explanation for this remarkable tendency in the folding of protein molecules may be given as follows: The regular conformations of α-helices and β-strands have considerably low energy-values[6], enhancing their probability of occurrence in protein molecules. But, while forming a globular shape in the acqueous medium the residues have to take up conformations other than α-helices and β-strands and remarkably enough, the percentage composition of these non-regular conformations is almost a constant (55%).

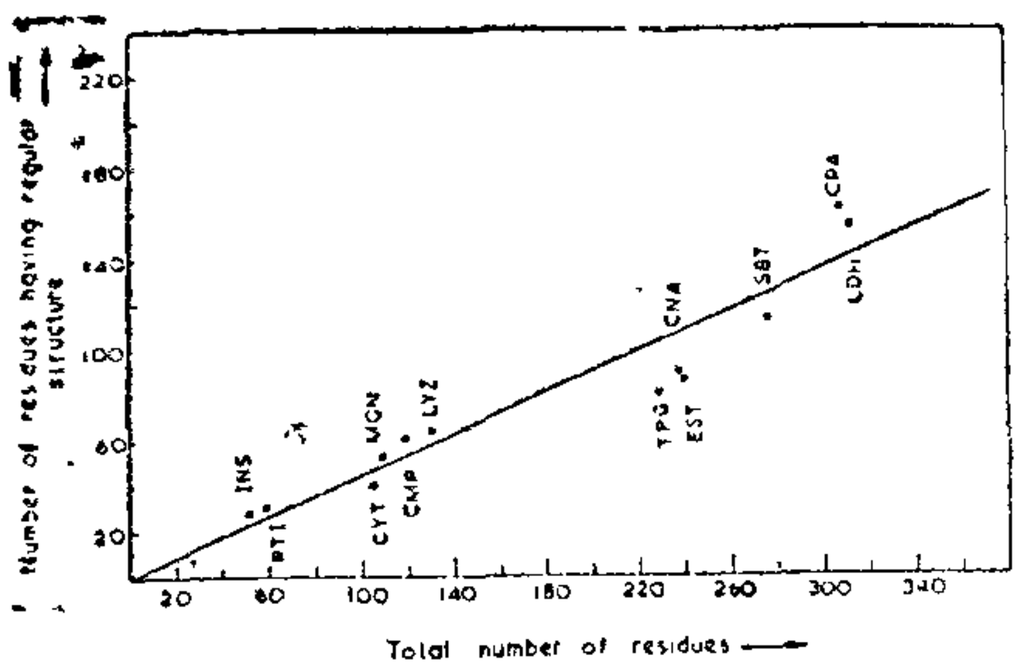

FIG. 1. The plot of number of residues in regular conformations *versus* the total number of residues, in various proteins. For details see text.

Of course there are a few exceptions, like myoglobin[7] which has about 80% of the residues forming a special nonpolar packet for the heme group and triose phosphate isomerase which has a $(\beta\alpha)_8$ barrel[8] consisting of 55% of α-helical residues and 22% of β-strands (a total of about 80% of residues in regular conformations). Further studies in these lines are in progress.

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SOME REACTIONS OF 3, 4-DICHLORO-COUMARINS

IN an earlier communication, we have described the syntheses of some 3, 4-dichlorocoumarins[1] by the reaction of hexachloropropene with phenolic compounds.

The present work reports some of the reactions of these halogenated coumarins.

The condensation of 6, 7-dimethyl-3, 4-dichlorocoumarin[1] (I) was effected with piperidine, morpholine, diethylamine and N-methylpiperizine by boiling them in alcoholic solution to yield the respective 4-amino derivatives (m.p. 174°; 167–168°; 145° and 152–153° respectively). The latter were tested at the Central Drug Research Institute, Lucknow, but none of them showed any appreciable antibacterial activity.

Also, (I) on condensation with ethanolamine in refluxing methanol gave 3-chloro-4-(2-hydroxyethylamino)-6, 7-dimethyl-coumarin as a colourless crystalline solid, m.p. 212–214° (yield 50%). Attempts to cyclise it to an oxazine derivative were futile. However, 6-bromo-3, 4-dichlorocoumarin[2] (II) with ethanolamine gave 3-chloro-4-(2-hydroxyethylamino)-6-bromocoumarin (III), (m.p. 198–199°, yield 40%), which was cyclised in the presence of sodium hydride to yield 9-bromo-3, 4-dihydro [1]-benzopyrano-[3, 4-*b*] [1, 4] oxazin-5-[1H]-one (IV), crystallised from acetic acid in pale brown plates, m.p. 295–296°.

The coumarin (I) was also condensed with *o*-phenylenediamine in N-methylpyrrolidone to yield 3-chloro-4-(*o*-amino-anilino)-6, 7-dimethylcoumarin (V) (m.p. 213–215°) which was cyclised by heating the latter with pyridine and MnO_2 to give 2, 3-dimethyl-6H-[1] benzopyrano (3, 4-*b*) quinoxalin-6-one (VI) crystallised from glacial acetic acid in yellow plates, m.p. 265°.