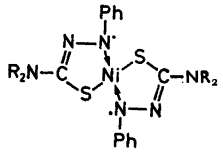
phenylazothioformamide by this method and shown that it possesses antifungal properties.

The phenylazothioformamides thus obtained react with nickel(0) compounds ($Ni(CO)_4$ or $Ni(P(OR)_3)_4$) with the formation of complex compounds which, according to appearance, analyses, and infrared spectra, are identical with those obtained by oxidation of the nickel(II) compounds. Considering the methods used for their preparation, the nickel complexes might be described as either nickel(IV) compounds, derived from the thiosemicarbazidate anion, or as nickel(0) compounds derived from the phenylazothioformamide. However, the properties of the compounds indicate that the most reasonable description of their constitution is as nickel(II) compounds derived from a thiosemicarbazidate anion radical:



For the nickel compounds extensive spin coupling of the unpaired electrons with the electrons of the unfilled *d*-shell of nickel(II) ion must take place, since the compounds are diamagnetic or only weakly paramagnetic. This is, of course, equivalent to saying that the formulation of the nickel compounds as nickel(IV) compounds, derived from the ligand ion L^{2-}, is not a bad approximation. However, the corresponding zinc compounds, even though resembling the nickel compounds very much in colour, are paramagnetic with a magnetic moment of approximately 2.4 B. M. In this the free electrons of the ligand cannot couple with unpaired electrons of the metal ion, accordingly, we actually are dealing with a zinc(II) complex of a radical ligand.

Nickel(II) compounds of thiopivaloylhydrazine and selenopivaloylhydrazine have been found to form similar strongly coloured complexes on oxidation.

The structure of the oxidized nickel compounds was confirmed by NMR spectroscopy. It has also been shown by polarography that they possess electron-transfer properties.

The details of this investigation will be published in a forthcoming paper in this journal.

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A Method for Determining Free Amino Groups in Polymers with Particular Reference to the Merrifield Synthesis

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For successful application of the solid-phase peptide synthesis method introduced by Merrifield [1] it is important to know the kinetics of the condensation reactions. The condensation reactions must go to completion, preferably at a high rate. Only one method has been described how to determine the free amino groups left, due to incomplete coupling.[2] We should like to publish another analytical procedure based on the condensation reaction of 2-hydroxy-1-naphthaldehyde with the free amino groups in the polymer. A quite stable aldimine (Schiff's base) is formed.[3] The chromophore is then displaced from the polymer by an amine, *e.g.* benzylamine, and the amount of the soluble aldimine thus formed is determined.

Procedure. 10 mg of polymer is allowed to react for 12 h with a great excess (50–100 times) of 2-hydroxy-1-naphthaldehyde at room temperature in absolute ethanol. After careful

washing of the polymer with methylene chloride, 2 ml 0.4 M benzylamine in methylene chloride is added and allowed to react for 30 min. The absorption at 420 nm is measured spectrophotometrically and the amount of 2-hydroxy-1-naphthalylidene-benzylamine is calculated. The contribution of the excess benzylamine to the absorption can be neglected.

Polymer-fixed peptide of the sequence, glycyl-leucyl-alanyl-valine, was chosen as model for study. The general method of synthesis was the following: The Boc-amino acid,* dissolved to 0.066 M in methylene chloride, was then added, 1.5 moles for each mole of amino acid or peptide bound to the polymer. Typically we used 0.66 mmoles of Boc-amino acid for about 2 g of polymer. After 3 to 5 min 0.66 mmoles of dicyclohexylcarbodiimide in methylene chloride (also 0.066 M) was introduced in the suspension. After specified times about 50 mg portions of polymer were removed, washed on a fritted glass filter with 1.5 ml 0.4 M benzylamine in methylene chloride (to stop the reaction) followed by absolute ethanol and finally with methylene chloride. For times shorter than 5 min the reaction was performed in a similar way but 50 mg portions of the polymer were allowed to react in a glass filter funnel for the time specified and the reaction was again stopped by adding benzylamine. After washing, drying and weighing, the content of residual amino groups was determined as described above. Unblocking of the Boc-group was achieved by treating the polymer with 1.1 M hydrogen chloride in glacial acetic acid.

Table 1 shows that the condensation of the Boc-amino acids in each step in the synthesis takes place rapidly and is completed in about 30 min.

In another experiment Boc-isoleucine was coupled to Val-polystyrene. Owing to sterical hindrance the reaction proceeded much slower in this case. About 10% of the original amino groups were still in free form after 21 h. This result is similar to that reported by Weygand and Obermeier for the corresponding reaction of Boc-isoleucine with Ile-Leu-Val-OCH_2-CO polystyrene. The reaction rate appears to be governed chiefly by the bulkiness of the carboxylic reactant. The results in Table 2 show a quantitative unblocking in each step by the treatment of Boc-amino acid or Boc-peptide-polystyrene with hydrogen chloride in glacial acetic acid.

* Boc=*tert*-Butyloxycarbonyl.

Table 1. Synthesis of the peptide glycyl-leucyl-alanyl-valine on polystyrene with carbodiimide.

No.	Carboxyl component	Reaction time [s]	Yield[a] [%]
1	Boc-alanine	28	25
2		30	27
3		58	61
4		145	90
5		300	99
6		1800	100
7	Boc-leucine	12	22
8		26	36
9		63	64
10		90	73
11		177	90
12		300	98
13		600	99
14		1800	100
15	Boc-glycine	26	46
16		58	62
17		118	68
18		195	99
19		300	100
20	Boc-isoleucine[b]	70[c]	32
21		300	72
22		600	78
23		1800	83
24		3600	84
25		21[d]	90

[a] All values corrected with regard to weight increase of the Boc-amino acid coupled.
[b] Boc-isoleucine coupled to Val-polystyrene.
[c] Polymer was removed from the bulk-suspension.
[d] Hours.

Table 2. Unblocking of the Boc-group with 1.1 M hydrogen chloride in glacial acetic acid.

No.	Amino acid- or peptide-polystyrene	Substitution[a] [μmoles/g Val-polystyrene]
1	Val-	267
2	Ala-Val-	261
3	Leu-Ala-Val-	258
4	Val-	236
5	Ala-Val-	235
6	Leu-Ala-Val-	235
7	Gly-Leu-Ala-Val-	241

[a] All values corrected with regard to weight increase.

The figures in Table 1 show small differences in the reaction rates for Boc-glycine, Boc-alanine, and Boc-leucine. The reaction rates for these three amino acids fall, however, in the expected order, taking into account the steric influences of the side chains.

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