Benzyl Cyanide, Benzyl Thiocyanate and Benzyl Isothiocyanate, Gas Chromatographic Data and Determination

Ö. WAHLROOS and MAIJA SAARIVIRTA

Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland

During an investigation of benzyl cyanide (BCN), benzyl thiocyanate (BTC), and benzyl isothiocyanate (BITC) formation in green plants and seeds [1,2] it became desirable to obtain a rapid method for quantitative evaluation of these substances. Ettlinger [3] has determined the corresponding allyl compounds on a capillary column coated with 1,3-benzenediacetonitrile.

For the quantitative analyses a Perkin Elmer 800 instrument equipped with a flame ionization detector and butanediol succinate polyester (BDS) stainless steel columns was used. Other data were obtained with a 1.8 m, 20 % polypropylene glycol column (R), and with a gas chromatograph constructed at this laboratory using an Apiezon L, Na-caproate column and an argon tetrode detector with the screen electrode and anode at 7 and 900 V, respectively.[4]

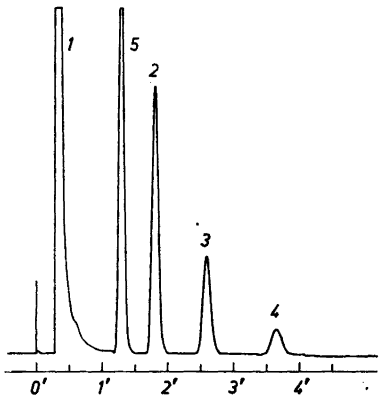

Fig. 1. 8 % wt BDS, silanized Chrom. W, 80–100, M, 1.8 m, 1.8 mm Ø, 190°C, 30 ml N_2/min. 1 solvent, 2 BCN, 3 BITC, 4 BTC, 5 anethole.

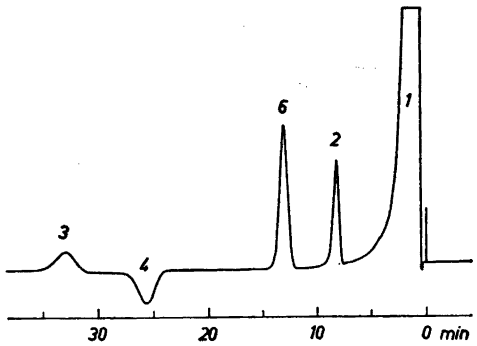

Fig. 2. 20 % wt Apiezon L, 1 % Na-caproate, sil. Chrom, P, 60–80 M, 1.8 m, 4 mm Ø, 200°C, 40 ml/min. Ar. 1,2,3,4 as before, 6 dodecane.

Retention times are shown in Figs. 1 and 2 and the Kováts indexes in Table 1. Anethole, used as an internal standard, separates poorly from BTC on Apiezon. The retention index given by Kováts [5] for BCN on a pure Apiezon L column is 1127 while the value obtained, when corrected for $\partial I/\partial T$, was 1117. It thus seems that the addition of Na-caproate does not increase the polarity of the station-

Table 1. Kováts retention indexes (I) and index increments on different columns.

	Api L, 200°C	BDS, 190°C		R, 190°C	
	I	I	ΔI	I	ΔI
BCN	1122	2362	1240	1419	297
BTC	1313	2918	1605	1612	299
BITC	1354	2656	1296	1634	280

Table 2. Comparison of retention index increments with boiling point difference on Api L column, 200°C.

	5 . Δ Bp	ΔI
BCN–BTC	10	191
BTC–BITC	35	41

Table 3. Elution temperatures on BDS column, program 30–200°C, 6.7°/min.

Allyl-CN	62
» ITC	83
» TC	98
Anethole	147
Benzyl-CN	154
» ITC	171
» TC	183

Table 4. Relative sensitivities. (Mass sens. to dodecane not identical in FID and argon.)

	FID	Argon
Dodecane	1	1
Anethole	1.1	
BCN	1.1	1.8
BTC	0.5	−1.3
BITC	0.7	0.4

ary phase. The index on polyethyleneglycol [5] was 1686, so that the polarity of this phase is between that of R and BDS. A comparison between the index increments on Apiezon and the boiling point differences (Table 2) reflects the fact that BTC and BITC are closer to each other than to BCN in polarity. Table 3 gives the elution temperatures by programmed temperature chromatography on BDS. The standard deviation for the elution temperatures is 1.3°C.

The approximative relative sensitivities (Table 4) were calculated from surface measurements, putting the sensitivity to dodecane arbitrarily = 1. The values illustrate the possibility of obtaining qualitative information by a combination of these two detectors. In the argon detector, BTC was ionized in an anomalous way, giving a negative signal. This was practically linear to a concentration of 0.05 μg/ml carrier gas. When the concentration rose to about 0.1 μg/ml, positive ions were apparently formed.

For quantitative determination, anethole was added as an internal standard to the ether extract of the water vapor distillate of the biological material. Peak areas were determined by weighing. The relative sensitivity (anethole = 1) needed for the calculation was obtained from the slope

Table 5. Change of the relative sensitivity (S) with regard to anethole with aging of the stainless steel-BDS column. FID.

Working hrs.	S		
	BCN	BITC	BTC
10	1.16	0.97	0.72
40	0.92	0.75	0.47
220	0.87	0.63	0.47

of the graphically determined regression line when peak area ratios from 16 chromatograms of known solutions containing various concentrations of benzyl compounds and anethole were plotted against the weight ratios. The relative sensitivities changed when the column was aged (Table 5). Possibly this is due to variation in the degree of decomposition of the benzyl compounds during chromatography. Rechromatography of the trapped front and back parts of the BTC peak indicated the formation of small amounts of decomposition products, about 4 %, with retentions 0.87 and 1.1, respectively, relative to BTC. The decomposition products are visible as a tailing of the peak, especially when small amounts are injected.

The standard deviation for peak area measurement was 3.6 % (2.4 cm^2 samples), for peak area + gas chromatography between 5 (BCN) and 7 % (BTC) when 2 μg was injected, and between 6.5 and 9 % when 0.02 μg was injected. For the whole analysis the S.D. was 10 % (0.08 mM) on the 0.8 mM/100 g level. The standard deviation for the gas chromatographic part of the analysis is surprisingly high. This is thought to be partly due to decomposition. Careful standardization of injector temperature could improve the precision. Partial decomposition was found to take place even at 150°C. During the slower elution at this temperature the products were separated to a greater extent from the mother peak than at 190°C. Better defined peaks were observed when temperature and gas flow rate were high. When the ether solution was concentrated ten times by distillation with a short fractionating column before chromatography, the standard deviation of the determination was lowered in consistency with the better instrumental precision when bigger samples were injected.

The research has been financed in part by a grant made by the *United States Department of Agriculture, Agricultural Research Service.*

1. Virtanen, A. I. and Saarivirta, M. *Suomen Kemistilehti* **B 35** (1962) 248.
2. Saarivirta, M. and Virtanen, A. I. *Acta Chem. Scand.* **17** (1963) *Suppl.* **1**, S 74.
3. Ettlinger, M. *Personal communication.*
4. Wahlroos, Ö. *Acta Chem. Scand.* **16** (1962) 1039.
5. Wehrli, A. and Kováts, E. *Helv. Chim. Acta* **42** (1959) 2709.

Received October 30, 1964.

Chemical Coupling of Amino Acids, Peptides and Proteins to Sephadex

ROLF AXÉN and JERKER PORATH

Institute of Biochemistry, University of Uppsala, Uppsala, Sweden

Immunosorbents may be synthesized by coupling antigens to solid hydrophilic supports.[1-4] The supports should be devoid of fixed ionic groups and other potential non-specific adsorption sites. Cross-linked dextrans (Sephadex) possess the required properties and have been used already as matrices for specific sorbents.[5]

A few years ago we fixed human and porcine blood group substances A on Sephadex G25 (Porath and Killander, unpublished) by diazo coupling with *p*-aminobenzyl Sephadex. Although the results were promising, the work was discontinued for several reasons. We are now following up our earlier investigations with a wider scope of application in mind and are exploring other methods of coupling.

Kent and Slade have suggested condensation of proteins with isothiocyanate fixed to a polymer (polyaminostyrene).[6] This communication describes the results of some exploratory studies using the mode of fixation suggested by Kent and Slade but with *p*-amino-phenoxy-hydroxypropyl and *β*-amino-ethyl ethers of Sephadex G25. These starting materials were kindly supplied by Mr. Björn Söderquist and Dr. Bertil Gelotte of Pharmacia, Uppsala.

The amino ethers were converted to the corresponding isothiocyanate derivatives by treatment with thiophosgene.

The amino derivatives of Sephadex were allowed to swell in potassium hydrogen phosphate buffer (3.5 M KH_2PO_4/K_2HPO_4, pH 6.8). A 10 % solution of thiophosgene in carbon tetrachloride was added (3 ml/g of amino derivative) and the reaction was carried out under vibration for 1 h. The gel was then washed for 5 min with 0.5 M sodium hydrogen carbonate, followed by sequential washings with water and portions of water-acetone-mixtures of increasing acetone concentrations to remove solids and to shrink the gel.

In order to estimate the isothiocyanate content a portion of the dried product was hydrolyzed with hydrochloric acid and the hydrogen sulphide formed was iodometrically determined.

The reactions between the isothiocyanate gel and the amino acids or amino acid derivatives were performed in aqueous sodium hydrogen carbonate solution or in formamide containing triethylamine. After mixing the suspension at room temperature for 20–30 h the gel was washed with 0.5 M sodium hydrogen carbonate, water, 0.5 M hydrochloric acid, with water-ethanol mixtures of increasing ethanol concentration, and finally, with pure ethanol.

In Table 1 are compiled relevant data for a number of experiments with *p*-isothiocyanato-phenoxy-hydroxypropyl Sephadex with an isothiocyanate-content of 120–150 *μ*equiv./g dry gel substance.

The amino acid analyses were performed according to Spackman, Moore and Stein using the Spinco automatic amino acid analyzer. To determine the effect of the carbohydrate on the recoveries of amino acids, mixtures of the free amino acids and Sephadex were "hydrolyzed" and analyzed. No losses were observed with the particular amino acids used in these experiments. Since, in the coupled products the amino acids presumably have been converted to substituted thioureas a quantitative reversion to amino acids upon hydrolysis cannot be expected. Therefore, analytical data in Table 1 are minimum estimates of the extent of substitution.