Hauser of the Massachusetts Institute of Technology, Drs. C. B. Stanwood and C. A. Turner, of the Great Northern Paper Company, all helped in the procurement and preparation of the wood meals. One of us (P. F. R.) wishes to express thanks to The D. S. and R. H. Gottesman Foundation of New York for a Scholarship and to the Canadian Pulp and Paper Association for a summer maintenance grant.

Summary

1. The composition of lignin as it exists in Northern Pine and Black Spruce wood was found to be about C, 67.5; H, 6%, by an indirect method that involved no assumptions concerning the chemical nature of the lignin. Methoxyl contents were calculated to be in the ranges 14.4 to 17.9% and 13.6 to 14.3%, respectively. The analyses were those of aromatic, rather than hydroaromatic or carbohydrate, substances.

2. Klason lignins isolated from the same woods had carbon and hydrogen analyses agreeing with the calculated values, provided the lignins were not dried by heating at 105°. When this precaution was neglected, the analyses were 2 to 5% low in carbon and resembled those published for most other isolated lignins. The methoxyl content of spruce, and perhaps of pine Klason lignin was about 1.5% higher than the calculated values. Most suggested structural formulas are too deficient in carbon content to represent lignin *in situ*.

3. Spruce or pine wood meal was alternately oxidized with aqueous sodium paraperiodate at *p*H 4 and 20° and boiled with water at *p*H 6.5 to dissolve the oxidized holocellulose. The residual, insoluble "periodate lignin" was only slowly attacked by the oxidant, contained no holocellulose and 86 to 96% of Klason lignin. Analysis showed that "periodate lignin" had undergone chemical change, presumably an oxidation rather than a resinification.

MONTREAL, QUEBEC, CANADA
RECEIVED DECEMBER 30, 1946

[CONTRIBUTION FROM THE ABBOTT LABORATORIES]

Some Schiff Bases of Free Amino Acids[1]

BY FLOYD C. MCINTIRE

Schiff base formation appears to be almost unrecognized among the various reactions between amino acids and aldehydes or ketones.[2] Although the esters and salts of various amino acids have long since been reported to form Schiff bases with aromatic aldehydes,[3] there are very few Schiff bases of the free amino acids on record. Bergmann and Zervas[4] reported the preparation of some monoarylidine derivatives of the diamino acids, but there appear to be no unequivocal reports of the preparation of Schiff bases of the free monoamino acids. Attempts to prepare these from the Schiff bases of the monoamino acid esters or salts have resulted in decomposition. Dakin[5] obtained preparations which had the correct analyses for benzylidineglycine, -alanine and -leucine, but he considered these preparations to be polymers. They were not crystalline and they were prepared under rather drastic conditions with very low yields. Gulland and Mead[6] studied amino acid Schiff base formation as related to *p*H in aqueous-alcohol solutions. They concluded that the Schiff base formation takes place primarily above *p*H 7 and is a reversible reaction so that the isolation of a Schiff base of a monoamino acid would be highly improbable.

The purpose of this paper is to report the preparation of some Schiff bases of free amino acids, particularly of the monoamino acids. These compounds have been prepared in crystalline form and in good yield by the reaction of amino acids with *o*-hydroxy aromatic aldehydes under very mild conditions. The conclusion that these compounds are actually Schiff bases is based upon the following criteria: (1) elementary analyses check well with the theoretical values; (2) representative members have been hydrolyzed under very mild conditions to yield the corresponding amino acids in good yield; (3) representative members have been hydrogenated to yield the corresponding N-arylamino acids.

Experimental

Materials.—2-Hydroxy-1-naphthaldehyde was prepared by the method of Duff and Bills.[7] 2-Methoxy-1-naphthaldehyde was prepared by methylation of 2-hydroxy-1-naphthaldehyde with dimethyl sulfate. Other aldehydes were purchased from Eastman Kodak Company.

All of the monoamino acids and glutamic acid were used in the free amino acid form.

The basic amino acids were prepared for use as follows: Lysine monohydrochloride was dissolved in water and shaken overnight with an excess of silver oxide. Most of the silver was removed from solution by precipitation with

(1) Presented in part before the Division of Biological Chemistry at the 110th meeting of the American Chemical Society, Chicago, September 9–13, 1946.

(2) Clarke, "Amino Acids" in "Organic Chemistry, An Advanced Treatise," 2nd ed., edited by Gilman, *et al.*, John Wiley and Sons, New York, N. Y., 1943.

(3) Bergmann, Ensslin and Zervas, *Ber.*, **58**, 1034 (1925); Gerngross, *Biochem. Z.*, **108**, 84 (1920); Gerngross and Zühlke, *Ber.*, **57**, 1482 (1924).

(4) Bergmann and Zervas, *Z. physiol. Chem.*, **152**, 282 (1926); **172**, 277 (1927).

(5) Dakin, *J. Biol. Chem.*, **82**, 439 (1929); **84**, 675 (1929).

(6) Gulland and Mead, *J. Chem. Soc.*, 210 (1935).

(7) Duff and Bills, *J. Chem. Soc.*, 1307 (1934).

an excess of carbon dioxide; the last traces of silver were removed with hydrogen sulfide. The solution was evaporated at reduced pressure and the residue was dried *in vacuo*. This residue was used directly for Schiff base formation. No attempt was made to determine the extent to which the lysine may have existed as the carbonate. Arginine monohydrochloride was dissolved in water and was treated with an excess of silver carbonate. The excess silver was precipitated by careful back titration with hydrochloric acid. The solution was evaporated at reduced pressure and the residue was dried *in vacuo*. Nitrogen analysis indicated that the preparation was approximately the half carbonate of arginine—$C_6H_{14}N_4O_2 \cdot 0.5H_2CO_3$. This preparation was used directly for Schiff base formation.

Preparation of the Schiff Bases.—The aromatic aldehyde was dissolved in absolute 3A alcohol (95% ethyl, 5% methyl). This solution was protected from moisture and stirred for twenty-four to forty-eight hours with the dry, finely powdered amino acid. The amino acid slowly dissolved as its reacted with the aldehyde. Ordinarily, 0.01 mole of amino acid, 0.015 mole of aldehyde and 300 cc. of alcohol were used. For complete reaction of a given quantity of amino acid, the necessary excess of aldehyde varied greatly, depending upon both the amino acid and the aldehyde. Some of the Schiff bases were not very soluble in cold alcohol and they began to crystallize out before the amino acid had completely dissolved. In such cases a larger portion of alcohol was used. The Schiff bases were obtained by concentrating the alcoholic solutions at reduced pressure to about 10 cc. and adding 50 cc. to 100 cc. of absolute ether. In some cases a few days in the cold room was required for maximum crystallization. The crystals were collected in centrifuge tubes, washed with ether and dried *in vacuo*.

All preparations here reported have been recrystallized from hot alcohol. With some Schiff bases it was necessary to add about a 50% excess of the corresponding aldehyde to the alcohol before recrystallization.

Hydrolysis of Schiff Bases and Recovery of the Amino Acids.—In 2 cc. of warm *N* hydrochloric acid in 50% alcohol was dissolved 0.6 to 1 millimole of the Schiff base. The color of the Schiff base disappeared after a few minutes at 60–70°. The solution was concentrated *in vacuo* to about 0.5 cc. The insoluble aldehyde was filtered off and washed. The filtrate and washings were concentrated to dryness *in vacuo*. The dry amino acid hydrochloride was dissolved in a few cc. of absolute alcohol and an excess of tributylamine was added. The free amino acid which crystallized out was collected by centrifugation, washed with absolute alcohol, and dried.

Hydrogenation of Schiff Bases

2-Hydroxy-1-naphthalglycine, 0.1 g., was dissolved in 100 cc. of methanol and reduced under 45 lb. hydrogen pressure, room temperature, twenty-four hours, with 0.5 g. of Raney nickel and 0.0125 mg. of chloroplatinic acid as catalyst. The Raney nickel was filtered off; water and hydrochloric acid were added to the methanol solution. The solution was warmed and concentrated to near dryness *in vacuo*. The sirup was dissolved in methanol containing excess hydrochloric acid. After decolorization with a few milligrams of Norit A the solution was concentrated to about 0.5 cc. and 10–15 cc. of absolute ether was added. The crystalline precipitate was washed with ether and dried *in vacuo*.

2-Hydroxy-1-naphthalglycylglycine, 0.4 g., was dissolved in 200 cc. of methanol and was reduced at room temperature, 45 lb. hydrogen pressure, with 0.1 g. of platinum oxide as catalyst. The reduction was compete in about five minutes. The reduction product was isolated as the hydrochloride by dissolving in methanol containing hydrochloric acid, concentrating the solution to about 15 cc., and then adding a few drops of concentrated hydrochloric acid and about 75 cc. of ether. After twenty-four hours in the cold room, the crystals were collected by centrifugation, washed with ether, and dried *in vacuo*.

2-Hydroxy-1-naphthalvaline, 0.5 g., was dissolved in 200 cc. of methanol and was hydrogenated under 45 lb. hydrogen pressure at room temperature, with 0.1 g. of platinum oxide as catalyst. The reduction went rapidly. The 2-hydroxy-1-naphthyvaline was isolated by the same procedure as with 2-hydroxy-1-naphthylglycylglycine.

Results and Discussion

The Schiff bases here reported are shown in Table I. These compounds usually crystallize as fine needles which are bright yellow in color. All are insoluble in water and soluble to some extent in ethyl and methyl alcohols. Some are much more soluble than others, depending upon both the aldehyde and the amino acid. Most of them are only slightly soluble in ether. In the melting point bath they decompose without melting sharply. The arginine derivative decomposed at 235–240°; all others decomposed in the range of 140–200°. No attempt was made to establish the constancy of their decomposition points through several recrystallizations. The stability of the Schiff bases of 2-hydroxy-1-naphthaldehyde varies with the amino acid. Some of them, *e.g.*, the valine derivative, can be recrystallized to a constant analysis from hot alcohol. Others will decompose during recrystallization unless an excess of the aldehyde is present. 2-Hydroxy-1-naphthalvaline appeared to be stable in 80% aqueous methanol at room temperature for several weeks.

TABLE I

FREE AMINO ACID SCHIFF BASES AND THEIR HYDROGENATION PRODUCTS

Schiff base	Yield, %	Empirical formula	Elementary analyses, %	Calcd.	Found
2-Hydroxy-1-naphthalglycine	62	$C_{13}H_{11}NO_3$	C,	68.1	67.82
			H,	4.81	4.79
			N,	6.11	6.04
alanine	76	$C_{14}H_{13}NO_3$	N,	5.76	5.76
phenylalanine	85	$C_{20}H_{17}NO_3$	N,	4.39	4.37
methionine	83	$C_{16}H_{17}NO_3S$	N,	4.66	4.36
valine	85	$C_{16}H_{17}NO_3$	C,	70.8	70.6
			H,	6.27	6.44
			N,	5.15	5.18
leucine	<50	$C_{17}H_{19}NO_3$	N,	4.91	4.79
isoleucine	90	$C_{17}H_{19}NO_3$	N,	4.91	4.83
glutamic acid	<50	$C_{16}H_{15}NO_5$	N,	4.65	4.75
lysine[a]	83	$C_{28}H_{26}N_2O_4$	N,	6.17	6.06
arginine[b]	70	$C_{17}H_{20}N_4O_3$	N,	17.05	16.06
glycylglycine	59	$C_{14}H_{14}N_2O_4$	N,	9.79	9.79
5-Chloro-2-hydroxy-1-benzylidinevaline	50	$C_{12}H_{14}ClNO_2$	N,	5.81	5.7
Hydrogenation product					
2-Hydroxy-1-naphthylglycine hydrochloride		$C_{13}H_{13}NO_3 \cdot HCl$	C,	58.2	58.22
			H,	5.22	5.25
			N,	5.22	5.06
glycylglycine hydrochloride		$C_{14}H_{16}N_2O_4 \cdot HCl$	C,	55.5	55.66
			H,	5.26	5.69
			N,	8.63	8.33
valine hydrochloride		$C_{16}H_{19}NO_3 \cdot HCl$	C,	70.3	69.4
			H,	6.96	7.0
			N,	5.13	5.13

[a] Nitrogen analysis was calculated for both amino groups in Schiff base formation. [b] Nitrogen analysis was calculated for only one amino group in Schiff base formation.

In the Schiff base formation some amino acids required a much greater excess of aldehyde than

did others. Valine, isoleucine and phenylalanine required less than a 50% excess of 2-hydroxy-1-naphthaldehyde to cause a given amount of the amino acid to dissolve completely, while leucine, methionine and alanine required about a 200% excess of the aldehyde.

In the reaction of glutamic acid with 2-hydroxy-1-naphthaldehyde, 1.5 moles of aldehyde were used per mole of glutamic acid in the reaction mixture. However, only 15% of the glutamic acid present was dissolved during the usual reaction time. This was interpreted as indicating that a 900 to 1000% excess of the aldehyde would be required to cause a given amount of glutamic acid to react completely. Since the Schiff base formation is reversed by acid conditions, this behavior of glutamic acid might be attributed to the presence of the second free carboxyl group.

Both amino groups of lysine reacted with 2-hydroxy-1-naphthaldehyde under the conditions of these experiments. Bergmann and Zervas used entirely different conditions and obtained monoarylidine lysine.

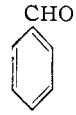
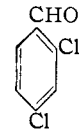
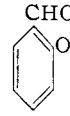
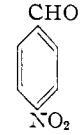
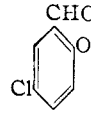
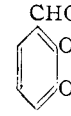
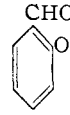
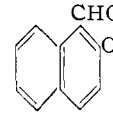
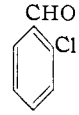
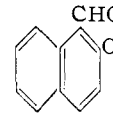
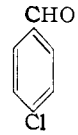
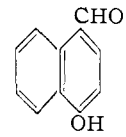
Although the high nitrogen analysis on the arginine derivative leaves some doubt as to the purity, and even the identity, of this compound, the data suggest that only one molecule of aldehyde condensed with each molecule of arginine. Whether free arginine would react more extensively with the aldehyde is yet to be determined.

Several different aromatic aldehydes were examined for Schiff base formation, but as indicated in Table II only the *o*-**hydroxyaldehydes** yielded Schiff bases which could be isolated. In order to study the relative tendency of different aldehydes to form Schiff bases with amino acids, a 50% excess of each aldehyde in absolute alcohol was stirred for twenty-four hours with a given quantity of valine. The amount of valine which dissolved was considered an indication of the relative tendency of the various aldehydes to form Schiff bases. From the reaction of various amino acids with 2-hydroxy-1-naphthaldehyde, valine appeared to be the amino acid of choice for this study. The data of Table II indicate that the *o*-**hydroxyaldehydes** showed by far the greatest tendency to react with valine. Methylation of the *o*-hydroxyl group, a shift of the hydroxyl group to the *para* position, or replacement of the *o*-hydroxyl by another strong negative group, *e.g.*, chlorine, resulted in compounds with relatively little tendency toward Schiff base formation. With certain aldehydes other than the *o*-hydroxyaldehydes an appreciable amount of valine was dissolved, but attempts to isolate the Schiff bases resulted only in the recovery of valine. In such cases any Schiff bases which may have been formed were too unstable to permit their isolation.

Of the three *o*-hydroxyaldehydes, salicylaldehyde dissolved the least valine. This Schiff base was isolated in very low yield and the analyses were not satisfactory. The substitution of a chlorine atom in the 5 position of salicylaldehyde resulted in a greater tendency to react with the amino acid and the corresponding Schiff base was stable enough to be isolated in 50% yield. The 2-hydroxy-1-naphthalvaline was isolated in 85% yield. Of these three aldehydes, 2-hydroxy-1-naphthaldehyde seemed to react most readily with the free amino acids and produced the most stable Schiff bases. In the reaction with isoleucine, 2-hydroxy-1-naphthaldehyde in 50% excess caused the amino acid to react completely and the corresponding Schiff base was stable enough to be recrystallized without an excess of aldehyde. A much larger excess of 5-chloro-2-hydroxybenzaldehyde was required for complete reaction of a given amount of isoleucine, and the product was not stable enough for recrystallization in the absence of an excess of aldehyde. These observations on Schiff base formation by the *o*-hydroxy aromatic aldehydes are in accord with the work of Vavon, *et al.*,[8] who showed that the *o*-hydroxyaldehydes react much more rapidly than the *m*- or *p*-hydroxyaldehydes in the formation of (1) oximes, (2) semicarbazones, (3) phenylhydrazones and (4) Schiff bases with *l*-menthylamine.

TABLE II

REACTION OF VALINE WITH DIFFERENT AROMATIC ALDEHYDES

Aldehyde	% Valine dissolved	Aldehyde	% Valine dissolved
	23		43
	92[a]		65
	100[a]		50
	20		100[a]
	34		24
	25		14

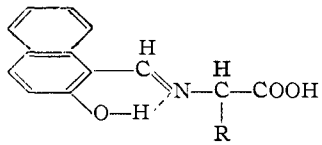
[a] These Schiff bases were isolated in crystalline form.

(8) Vavon and Anziani, *Bull. soc. chim.*, **4**, 2026 (1937); Vavon and Montheard, *ibid.*, **7**, 551, 560 (1940).

Hydrogenation of the —C=N— linkage was carried out on three of the Schiff bases as indicated in Table I. All three hydrogenated products gave positive ferric chloride tests for phenolic groups. The analyses suggest that with 2-hydroxy-1-naphthylglycylglycine the naphthalene ring may have been hydrogenated to some extent.

For —C=N— linkages of Schiff bases, these hydrogenations required extremely large amounts of catalyst. The glycine Schiff base was not noticeably reduced when 0.1 g. of 20% palladium on charcoal was used per 0.1 g. of Schiff base. The glycylglycine Schiff base was not noticeably reduced when five times its weight of Raney nickel with chloroplatinic acid promoter was employed.

This resistance to hydrogenation, along with the unusual ability of the **o-hydroxyaldehydes** to form Schiff bases with the monoamino acids, can be explained on a basis of intramolecular hydrogen bonding



This explanation is strengthened by analogy with salicylaldehyde anil which, according to Pauling,[9] has a very strong intramolecular hydrogen bond.

(9) Pauling, "Nature of the Chemical Bond," 2nd ed., Cornell University Press, Ithaca, N. Y., 1945, p. 319.

Upon hydrolysis of 2-hydroxy-1-naphthalvaline, -alanine and -methionine, as outlined above, the amino acids were recovered in 92, 80 and 75% yields, respectively.

2-Hydroxy-1-naphthaldehyde has been used as an amino group reagent in the isolation of amino sugars.[10] The stability of the amino acid Schiff bases and their ease of hydrolysis suggest the possibility of extending the use of *o*-hydroxy aromatic aldehydes as amino group reagents in biochemical isolation procedures.

Acknowledgment.—The author is grateful to E. F. Shelberg and associates for micro-elementary analyses, to Morris Freifelder for carrying out the hydrogenations and to J. R. Schenck for his interest and suggestions.

Summary

A number of crystalline Schiff bases of free amino acids, particularly the monoamino acids, were prepared by the reaction of free amino acids with *o*-hydroxy aromatic aldehydes under very mild conditions. Their stability is explained on a basis of intramolecular hydrogen bond formation. This explanation is supported by the fact that they are very difficult to hydrogenate. These Schiff bases can be hydrolyzed under mild conditions to give a good yield of the original amino acids.

(10) Jolles and Morgan, *Biochem. J.*, **34**, 1183 (1940).

NORTH CHICAGO, ILLINOIS RECEIVED FEBRUARY 4, 1947

[CONTRIBUTION FROM THE RESEARCH AND DEVELOPMENT DIVISION, COMMERCIAL SOLVENTS CORPORATION]

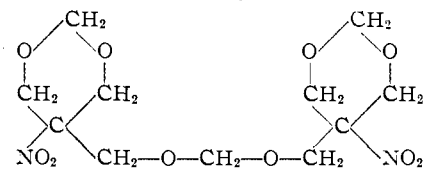
Acetals of Nitro Alcohols and Corresponding Amino Acetals[1]

BY MURRAY SENKUS

The preparation of cyclic acetals from polyhydric nitro alcohols derivable from formaldehyde and primary nitroparaffins has been reported in previous communications from this Laboratory.[2] We now wish to report the preparation of acetals from some monohydric nitro alcohols.

Adkins and Wade Adams had made a study of the catalysis of the formation of acetals from monohydric alcohols and aldehydes.[3] They found that calcium chloride is the most effective catalyst with the lower aldehydes and alcohols while dry hydrogen chloride is best for the higher members of these series. These catalysts were found to be unsuitable for the preparation of acetals from monohydric nitro alcohols owing to the insolubility of calcium chloride in the reaction mixtures used and the volatility of hydrogen chloride during the reactions. Benzenesulfonic acid and *p*-toluenesulfonic acid were found to be most satisfactory for the reactions at hand.

We also wish to report the preparation of 1,5-bis-(1-nitro-3,5-dioxacyclohexyl)-2,4-dioxapentane, from tris-(hydroxymethyl)-nitromethane



and formaldehyde. The structure of this compound is supported by nitrogen analysis and molecular weight determination and by the observation that three moles of formaldehyde reacted with two moles of tris-(hydroxymethyl)-nitromethane to give the calculated amount of product. Analytical data on the diamine obtained by hydrogenation of this compound also support the proposed structure.

(1) Prepared for the 1946 Fall meeting of the Organic Division, A. C. S. The subject matter of this paper is covered by U. S. Patents 2,415,046, 2,363,464, 2,413,249 and 2,413,250.

(2) (a) Senkus, THIS JOURNAL, **63**, 265 (1941); (b) **65**, 1656 (1943).

(3) Adkins and Wade Adams, *ibid.*, **47**, 1358 (1925).