[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

The Browning Reaction of Sugars and Amino Acids Approached by Means of Simple Hydroxy Ketones[1]

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Benzoin reacts with benzylamine or pentylamine at 100° to yield α-(benzylamino)-desoxybenzoin and α-(pentylamino)-desoxybenzoin, respectively, whereas at 170° benzylamine gives rise to N,N'-dibenzyl-1,2-diphenyl-1,2-ethanediimine, the latter compound also being formed by reaction of benzil and benzylamine together with some concurrent oxidation of benzylamine to the aldehyde state. Some oxidation of benzoin to benzil occurs on heating benzoin with 2-aminoethanol. The reaction of benzoin with α-amino acids at 165–175° causes evolution of carbon dioxide, formation of tetraphenylpyrrole by an aldolization process, and of tetraphenylpyrazine by an oxidation process. Benzoin, acetophenone and glycine at 175° yield 1-methyl-2,3,5-triphenylpyrrole. Acetol and phenacyl alcohol (both primary alcohols) react with α-amino acids to form brown products preceded by yellow and orange stages, and pyrazines are obtainable from the products. Acetoin (a secondary alcohol) reacts more slowly but both yellow and brown stages are reached; propionoin does not react beyond the orange stage. Oxidation of the brown polymers from acetol–phenylalanine revealed a 4:1 ratio of acetol to amino acid residues in the polymer. The general problem of browning of carbohydrates by amino acids or proteins is considered and an explanation is offered based in large measure on results with the model systems. The steps proposed to explain the colored bodies involve an Amadori rearrangement of aldose to the N-analog of the ketose, aldolization and/or oxidation to yield polymers containing cumulative carbonyl groups (α-diketones, triketones, etc.).

The excellent survey by Hodge[2] of the chemistry of non-enzymatic browning in sugar–amine systems summarizes the evidence for current thinking that the initial colorless stage is condensation of the amine or amino acid with the carbonyl group of the aldose followed by an Amadori rearrangement to the nitrogen-containing ketose isomer. In Hodge's opinion the next stage is either dehydration to furaldehydes or reductones or fragmentation of the sugar moiety or Strecker degradation of the amino acid moiety. In the final stage "the intermediates polymerize and unsaturated, fluorescent, colored polymers are formed. The chief reactions involved are thought to be aldol condensation, aldehyde–amine polymerization and formation of heterocyclic nitrogen compounds."

Thus, it is recognized that the final brown products must be high polymers. Since they are colored it seems obvious that they must contain chromophoric groups. Indeed, in Maillard's early paper[3] one finds the report of yellow and orange colors (with glucose, glycine and water) preceding the final brown, or wine red (with xylose) preceding the brown. In our work with simple hydroxy ketones and amino acids given below it will be shown that there is yellowing before browning. No suggestion regarding the type of chromophores or their mode of formation in the polymers has ever been made. Aldol condensations have been suggested as important in the final stages of browning. We suggest that condensations of the aldol type offer an excellent means of explaining these chromophores; also, we propose that aldolization occurs prominently in the intermediate as well as the final stages.

In our present work these simple hydroxy ketones were studied: acetol, phenacyl alcohol, acetoin, propionoin, benzoin (also benzil). This extends our earlier work[4] wherein it was shown that 2,3,4-trideoxypentose (I), $HOCH_2CH_2CH_2CH_2CHO$, gives no browning in its reaction with glycine whereas 3,4-dideoxypentose (II), $HOCH_2CH_2CH_2$-$CHOHCHO$, browns strongly, more so even than glucose. The aliphatic compounds in the present list have not been studied in this way, but a considerable literature does exist for benzoin.

Benzoin has been heated with aniline alone[5] to yield the anil, or with aryl-[6] or alkyl-ammonium[7,8] salts to yield amino ketones, the amine residue being attached to the carbon which was originally attached to the carbonyl group: $ArCH(NHC_6H_5)$-$COPh$ from $ArCOCHOHPh$. Some benzil was formed[7] as well.

This type of change resembles the Amadori rearrangement[9,10] of aldoses, wherein the glycosylamine formed from an aldose and an arylamine rearranges into the ketose isomer ($-CHOH-CH=NAr \rightarrow -CO-CH_2NHAr$), or the imine from a ketose rearranges into the aldose isomer[11] ($-C(=NEt)-CH_2OH \rightarrow -CHNHEt-CHO$) thus demonstrating a preferred trend toward formation of the oxygen-containing carbonyl group ($=C=O$ rather than $=C=NR$).

Since the Amadori rearrangement has been suggested as an intermediate stage in the browning process, it is pertinent to note that the formation of a Schiff base is also the first necessary step[12] in the Strecker degradation of an α-amino acid in the presence of $-CO-(CH=CH)_n-CO-$ groups, as exemplified with alanine and isatin

$R_2C=O$ (isatin) $\longrightarrow R_2C=N-CH(CH_3)COOH \longrightarrow$

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(2) J. E. Hodge, *Agr. Food Chem.*, **1**, 928 (1953).

(3) L. C. Maillard, *Ann. chim.*, [9] **5**, 258 (1916).

(4) C. D. Hurd and C. D. Kelso, THIS JOURNAL, **70**, 1484 (1948).

(5) C. N. Cameron, *Trans. Roy. Soc. Canada*, [3] **23**, Sect. 3, 53 (1929); **25**, Sect. 3, 145 (1931).

(6) K. Voight, *J. prakt. Chem.*, [2] **34**, 2 (1886); R. M. Cowper and T. S. Stevens, *J. Chem. Soc.*, 347 (1940).

(7) R. E. Lutz, J. A. Freek and R. S. Murphey, THIS JOURNAL, **70**, 2015 (1948).

(8) W. L. Truett and W. N. Moulton, *ibid.*, **73**, 5913 (1951).

(9) F. Weygand, *Ber.*, **73**, 1259 (1940).

(10) L. I. Smith and R. H. Anderson, *J. Org. Chem.*, **16**, 963 (1951); R. H. Anderson, *ibid.*, **19**, 1238 (1954).

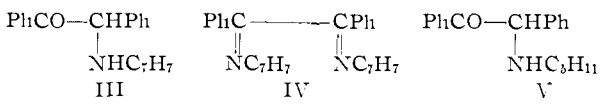
(11) (a) J. F. Carson, THIS JOURNAL, **77**, 1881, 5957 (1955); (b) **75**, 4337 (1953).

(12) A. Schönberg and R. Moubacher, *Chem. Revs.*, **50**, 261 (1950)

$$R_2CH{-}N{=}C(CH_3)COOH \xrightarrow{-CO_2}$$

$$R_2CH{-}N{=}CHCH_3 \xrightarrow{H_2O} CH_3CHO + R_2CHNH_2$$

In our work on the reaction of simple hydroxy ketones and α-amino acids or amines, we began with benzoin, benzylamine and no acid catalyst. At 100 or 150° the product was α-(benzylamino)-desoxybenzoin (III), a result which presumably involved initial attack at the carbonyl group, then rearrangement. Pentylamine reacted similarly at 100° to form the amino ketone V.



III IV V

If the heating with benzylamine was performed at 170° or if III was heated with benzylamine at 170°, then diimine IV was obtained, which yielded benzil on acid hydrolysis. IV was obtainable also from benzil by reaction with benzylamine at 150°, along with other products mentioned below. There was benzil production at 100° when 2-aminoethanol was heated with benzoin. These facts suggest that the initial product of the benzoin–amine interaction, PhC(=NR)CHOHPh, undergoes two competing reactions: (1) internal oxidation–reduction leading to structures like III, (2) bimolecular reactions with either III or benzoin leading either to benzil or its imino analog, such as

$$2PhC(=NR)CHOHPh \longrightarrow PhC(=NR)COPh + PhCH(NHR)CHOHPh$$

Evidence for the reversibility of a very similar reaction was that involving interaction of benzil and benzylamine at 150° wherein benzaldehyde (or its imino precursor) was found among the products

$$PhCOCOPh + PhCH_2NH_2 \rightleftharpoons PhCOCHOHPh + PhCH{=}NH$$

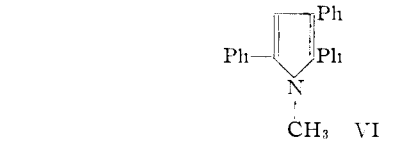
Lutz and co-workers[7] also observed benzil in their study of benzoin and alkylammonium salts.

An alternative explanation of the formation of benzil is rearrangement of III to Ph–CHOH–CPh=NC_7H_7, then oxidation of the latter with air, since a similar course of reaction has been reported[11b] between acetoin and cyclohexylamine or aniline. This explanation seems less attractive than the one cited because many of the experiments were conducted in an atmosphere of nitrogen, although it must be admitted that air was accessible during the crystallization procedure.

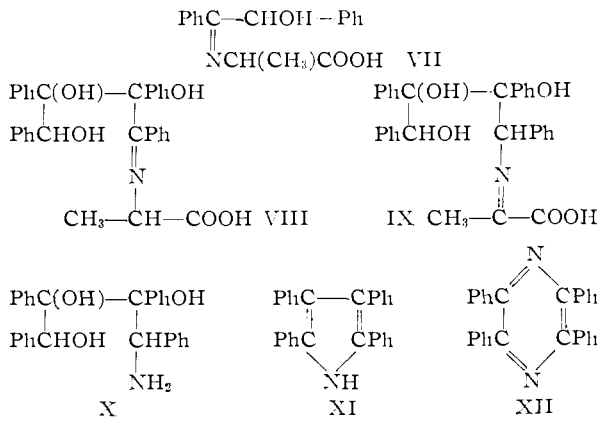
In the reaction of benzoin with amino acids (glycine, alanine, leucine) at 165–175°, evolution of carbon dioxide and formation of tetraphenylpyrazine was noticed in all experiments. Tetraphenylpyrrole may have been formed in all too, but it was actually observed only in the experiments with alanine where an intensive search was made for it.

A mixture of benzoin, acetophenone and glycine, treated similarly at 175°, yielded 1-methyl-2,3,5-triphenylpyrrole (VI), the structure of which was verified by synthesis from 2,3,5-triphenylpyrrole. It is evident that an aldol condensation is involved in forming VI from benzoin and acetophenone.

The importance of the pyrroles in browning studies is not because of the heterocyclic formation involved but rather because of the aldol condensation. These steps are suggested with benzoin: reaction with the amino acid giving VII and subsequent aldol condensation with benzoin yielding VIII (but it is conceivable that an aldol condensation with 2 moles of benzoin occurs first, followed by reaction with alanine to yield VIII). The change of VIII into IX involves tautomerism as in the Strecker degradation. This would be followed by hydrolytic and pyrolytic cleavage into CO_2, CH_3-CHO and X, finally ring closure and dehydration to XI (but some of the dehydration may have occurred earlier).



VI



VII VIII IX

X XI XII

The tetraphenylpyrazine (XII) which was observed obviously did not arise as a result of aldolization, but it does point to benzil or its imino equivalent as an intermediate.

As benzoin was found to undergo both aldolization (to pyrroles) and oxidation (to benzil or tetraphenylpyrazine), it also is reasonable to suggest that both aldolizations and oxidations should be encountered with the aliphatic α-hydroxy ketones. The greater reactivity of alkylpyrroles would prevent their isolation under the experimental conditions required for their formation, whereas the lesser reactivity of alkylpyrazines would permit their isolation, once formed. Three kinds of evidence make this assumption reasonable. (1) The color of the intermediate stages in the reaction of acetoin with alanine at 140° was yellow or orange, but the final stage was brown. (2) 2,4-Dimethylpyrrole, a probable intermediate from acetol and alanine, was found to brown extensively with acetol, hence its instability in the presence of one of the original reactants might account for its non-isolation. (3) Pyrazines were isolated from acetol, phenacyl alcohol or acetoin, the same as from benzoin, and the inertness of dimethylpyrazine (from acetol) toward acetol was demonstrated. Except to designate α-diketone precursors, the pyrazines would thus seem to be of no importance in the steps leading to browning.

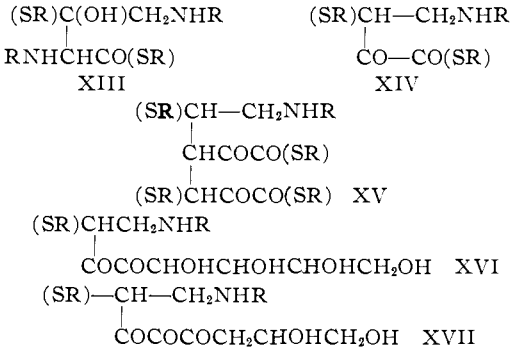
Brown products were always formed when acetol or phenacyl alcohol (and also glycolaldehyde) were

treated with amino acids. Often the brown appeared so rapidly as to mask the initial yellow stage, but the early color of the reaction of acetol with alanine (120°) was vivid yellow. 2,5-Dimethylpyrazine was found, but no dimethylpyrrole was isolable. The absorption spectrum of the dimethylpyrrole was isolable. The absorption spectrum of the dimethylpyrrole–acetol product, although similar to that obtained from acetol and alanine (Fig. 1) was not identical to it.

2,5-Diphenylpyrazine was obtained following the reaction of phenacyl alcohol and alanine. The chief product was a brown polymer. Information regarding the brown polymers was obtained in the reaction of acetol with β-phenylalanine. After removal of unused reactants from the brown mass it was oxidized. The quantity of benzoic acid obtained revealed a 4:1 ratio of acetol to amino acid residues in the polymer.

It is suggested that the two distinctive features found in the present work, namely, aldolization and oxidation, are also the two fundamental processes in the browning of sugars. Although the temperatures (100–170°) used with benzoin were hotter than is usual for the browning of aldoses, lower temperatures were found applicable for the aliphatic analogs and the rates of color formation at the temperatures used were comparable to those found when glucose was treated similarly.

In the formulations below, let (SR) represent "sugar residue," and let R represent an amino acid residue or other organic radical. Then let us assume initial formation of a cyclic or acyclic glycosylamine (A), (SR)–CHOH–CH=NR, which will undergo the Amadori change into B, (SR)–CO–CH_2NHR. Then aldolization of 2B would yield XIII which could dehydrate (and hydrolyze) into the yellow α-diketone XIV. Two more aldolizations of this with B would yield XV as well as other isomers.



Diketones could arise also by similar aldol condensations of B with A but not from 2A. Vicinal triketones, as XVII, can be visualized as coming from XIV by elimination of the elements of water in the manner of aldols, since the expanded form (XVI) of XIV shows that it is an aldol.

It is obvious that cyclizations of these intermediates to furans or pyrroles could occur readily. It is evident also that any furfural, pyruvaldehyde or reductone that may have been formed prior to aldols of the type mentioned above could readily condense with B to yield the same kind of polyketones.

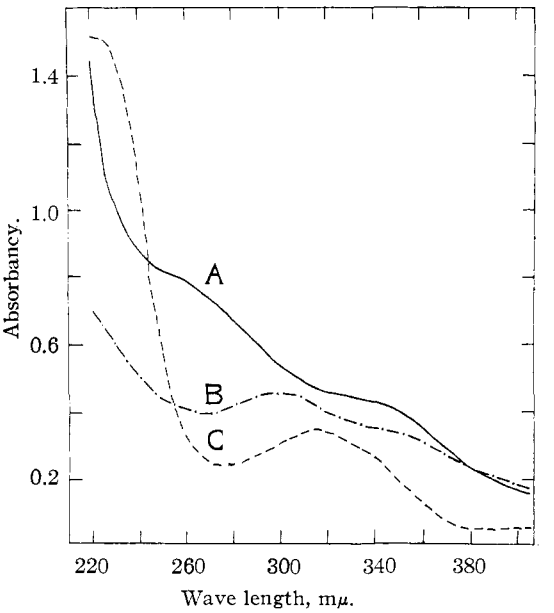
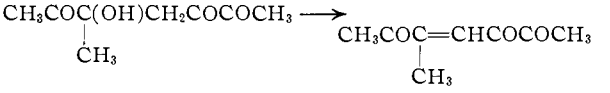


Fig. 1.—Absorption spectra of brown products from acetol–β-phenylalanine (A), acetol–alanine (B) and acetol–2,4-dimethylpyrrole (C).

The fact that a pyrazine was formed in the reaction of acetoin with alanine indicates an oxidation of the acetoin either to biacetyl or to some imino analog. It is suggested that the portion of the oxidized compound which escaped pyrazine formation underwent aldolization; if so, a vinylog of an α-triketone would be the first step in a condensation leading to brown colored bodies



The color change of the reaction was a gradual yellowing and finally browning, but this was a considerably slower process than was observed with the α-hydroxy ketones related to primary alcohols (acetol, phenacyl alcohol, glycolaldehyde). This is reasonable in view of the fact that aldolization of the acetoin could not yield colored compounds in the absence of an oxidation process. The color change with propionoin was still slower, showing an ultimate orange coloration but not brown.

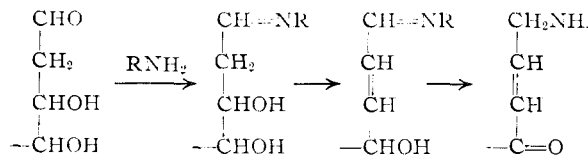
It is known[13] that dilute caustic solutions promote a change in color of biacetyl solution from yellow to brown as aldolization progresses.

Lea[14] observed that 2-deoxygalactose browned rapidly in the presence of casein (*p*H 6.3, 37°, rel. humidity 70%) and regarded it as unexpected in view of the reported non-browning of 2,3,4-trideoxypentose (I), which also lacks a hydroxyl group on carbon 2. The deoxygalactose is an aldol, however. Although it has no possibility of undergoing a simple Amadori rearrangement into a ketose, it can undergo dehydration in the manner of aldols into an unsaturated aldehyde, which could then

(13) H. von Pechmann, *Ber.*, **21**, 1419 (1888); O. Diels, W. Blanchard and H. v. d. Heyden, *ibid.*, **47**, 2356 (1914).

(14) C. H. Lea and D. N. Rhoades, *Biochem. Biophys. Acta*, **9**, 56 (1952); C. H. Lea, D. N. Rhoades and S. Borrell, *Nature*, **169**, 1097 (1952).

undergo an "extended" Amadori rearrangement and isomerize into the vinylog of a ketose



Such a compound now could undergo aldol condensations to yield polymers containing the di- or triketone functions which are considered to be responsible for color.

Experimental

Reactions of Benzoin. With Benzylamine at 170°.—A mixture of 1.1 g. of benzoin and 3 ml. of benzylamine was heated at 165–170° for 30 min., then was cooled and diluted with an equal volume of absolute ethanol. Yellow crystals (0.6 g., m.p. 90–94°) separated after 2 days. These became colorless after crystallization from absolute alcohol and melted sharply at 95–96°. Analysis was satisfactory for N,N'-dibenzyl-1,2-diphenyl-1,2-ethanediimine (IV).

Anal. Calcd. for $C_{28}H_{24}N_2$: C, 86.6; H, 6.23; N, 7.21. Calcd. for $C_{28}H_{26}N_2$: C, 86.1; H, 6.71; N, 7.17. Found: C, 86.8, 86.6; H, 6.11, 6.33; N, 7.67, 7.38.

A 455-mg. sample of the diimine was warmed with 2 ml. of concd. hydrochloric acid on a steam-bath. A yellow oil separated which crystallized on cooling; yield 170 mg. This was benzil, m.p. and mixed m.p. 93–94°. The m.p. was depressed to 80–84° when mixed with the original diimine.

With Benzylamine at 100°.—A mixture of 4.2 g. of benzoin and 2.1 g. of benzylamine was fused over a free flame, then was heated at 100° for 15 min. The oil was taken up in 10 ml. of absolute ethanol, from which 3.5 g. of crystals, m.p. 64–67°, separated on cooling. Two recrystallizations from ethanol yielded 1.35 g. of α-(benzylamino)-desoxybenzoin (III), prisms of m.p. 74–75° which were colorless but which acquired a yellow color on standing.

Anal. Calcd. for $C_{21}H_{19}NO$: C, 83.7; H, 6.35; N, 4.63. Found: C, 83.8; H, 6.63; N, 5.00.

V also was obtained by performing the experiment at 150° for 30 min. (using, however, 7 ml. of the amine instead of 2). Treatment of the compound with hydrochloric acid yielded the hydrochloride, m.p. 218–221° after crystallization. Analysis showed 4.58% N, compared to the calculated 4.41. This salt was previously reported by Lutz,[7] m.p. 219–222°.

A mixture of 1 g. of III and 3 ml. of benzylamine was heated in a bath at 170° under reflux for 30 min. Water was formed and it was permitted to escape. A viscous oil resulted on cooling. It crystallized from absolute ethanol, yielding 0.42 g. of IV, m.p. and mixed m.p. 95–96°.

Benzil and Benzylamine.—A mixture of 4.2 g. of benzil and 8 ml. of benzylamine was heated under nitrogen for 30 min. at 150°. When cooled, the mixture was washed with water and the undissolved portion was taken up in hot 95% ethanol. There appeared 2.3 g. of yellow solid, m.p. 92–94°, on cooling. Recrystallization from absolute ethanol yielded 2 g. of colorless IV, m.p. 96–97°. A mixture of this material with the diimine of m.p. 95–96° prepared from benzoin also melted at 96–97°.

A duplicate experiment was performed and the crude product was diluted with water, acidified and distilled to collect 25 ml. of distillate. The latter was added to a solution of 2 g. of 2,4-dinitrophenylhydrazine, 1 ml. of concd. hydrochloric acid and 100 ml. of alcohol. Benzaldehyde 2,4-dinitrophenylhydrazone, m.p. 237–238°, separated; yield 2.4 g. In a similar experiment but differing in that the diluted reaction mixture was distilled without acidification, there was obtained 0.25 g. of benzaldehyde 2,4-dinitrophenylhydrazone.

Benzoin with *n*-Pentylamine.—A mixture of 4.2 g. of benzoin and 5 ml. of pentylamine was heated for 15 min. on a steam-bath, then was dissolved in 20 ml. of benzene and washed with 40 ml. of 5% hydrochloric acid, thereby causing an orange coloration and the gradual separation of 5.4 g. of a salt melting approximately at 200°. It analyzed correctly for α-(pentylamino)-desoxybenzoin hydrochloride.

Anal. Calcd. for $C_{19}H_{24}ClNO$: Cl, 11.16. Found: Cl, 11.12.

Benzoin and 2-Aminoethanol.—Equal weights (4 g. each) of these two reagents were heated under nitrogen at 100° for an hour. The product was dissolved in 25 ml. of 95% ethanol, and to this mixture was added 14 ml. of concd. hydrochloric acid dropwise and with cooling. The color became dark red. On dilution, 0.42 g. of yellow crystals of benzil separated; m.p. and mixed m.p. 93–94° after recrystallization.

With Glycine.—Heating of a mixture of 4.2 g. of benzoin and 1.5 g. of glycine under carbon dioxide at 160–165° for 45 min. gradually caused disappearance of all the glycine and the production of a clear red-brown solution (with preceding yellow and orange colors). The mixture was cooled to 80° and absolute alcohol was added. The fine yellow crystals (0.12 g.) which separated were insoluble in hot, dilute solutions of sodium hydroxide or hydrochloric acid. The m.p. was 248–249°, and it was not depressed when mixed with authentic tetraphenylpyrazine of m.p. 250°.

In a comparable experiment it was demonstrated that the volatile products contained carbon dioxide but no aldehyde, ketone, amine or ammonia.

With Alanine.—A mixture of 8.5 g. of benzoin and 3.6 g. of DL-alanine was heated at 170–175° (or at 150°) for 1 hr. Volatile products were passed (nitrogen sweep) through a solution containing 0.25 g. of 2,4-dinitrophenylhydrazine, 0.4 ml. of concd. hydrochloric acid and 25 ml. of 95% ethanol. There separated 0.2 g. of acetaldehyde 2,4-dinitrophenylhydrazone (yellow plates, m.p. 161–162° from a mixture of ethyl alcohol and ethyl acetate). The reaction gas, when passed through barium hydroxide solution, caused precipitation of barium carbonate.

The non-volatile sirup in the reaction tube was dissolved in 15 ml. of warm glacial acetic acid. After several hours the precipitate (4.2 g.) was collected. After several fractional crystallizations from glacial acetic acid this precipitate was separated into the more soluble 2,3,4,5-tetraphenylpyrrole (XI) and the less soluble tetraphenylpyrazine (XII) in amounts of 1.26 and 0.66 g., respectively. The melting point (and mixed m.p.)[15] of XI was 215–216°; that of XII including mixed m.p.[16] was 253–254°. When XI was fairly well separated from XII, it was crystallized to purity from 95% ethanol. *Anal.* Calcd. for $C_{28}H_{21}N$: N, 3.77. Found: N, 3.93.

With Leucine.—There was obtained 0.03 g. of yellow needles, m.p. 249–250°, from a mixture of 2.1 g. of benzoin and 1.3 g. of DL-leucine, heated for 3 hr. at 165–170° and then diluted with 5 ml. of glacial acetic acid. Mixed m.p. determination confirmed its identity as tetraphenylpyrazine. The remainder of the reaction product was not processed.

With Glycine and Acetophenone.—One-hundredth molar quantities of benzoin, glycine and acetophenone were heated for 30 min. at 175° to yield a clear orange melt. After mixing this with 10 ml. of absolute ethanol there separated 0.54 g. of yellowish needles which became colorless after crystallization from glacial acetic acid and from ethanol; m.p. 177–178°. This proved to be the same substance as the methylation product VI of triphenylpyrrole.

To a solution of sodium *t*-butoxide (from 0.40 g. of sodium) in 40 ml. of *t*-butyl alcohol was added 0.29 g. of 2,3,5-triphenylpyrrole. Then, addition of 1 ml. of methyl sulfate caused immediate separation of sodium methyl sulfate. The mixture was refluxed for 15 min., poured into water, filtered and the solid on the filter was washed with methanol; yield 0.16 g., m.p. 160–167°. After recrystallization from absolute ethanol the colorless needles melted at 176–177°, and a mixture of it with that prepared from glycine melted at 176–177°. The sample from glycine was analyzed.

Anal. Calcd. for $C_{23}H_{19}N$: C, 89.3; H, 6.19; N, 4.53. Found: C, 89.5; H, 6.04; N, 4.90.

Reaction of Acetoin and Alanine.—Freshly distilled acetoin (2.5 g.) was added to 4.0 g. of DL-alanine in a flask flushed out with nitrogen. The mixture was refluxed (bath temp. 140°) for 1 hr. during which time the color gradually turned yellow and finally brown. Trituration of the cooled residue with ether extracted a fraction which was distilled at 55–90° (5 mm.); yield 0.82 g. Following the rather unsatisfactory method of Piloty and Hirsch[17] for separation of tetra-

(15) D. Davidson, *J. Org. Chem.*, **3**, 361 (1938).

(16) D. Davidson, M. Weiss and M. Jelling, *ibid.*, **2**, 328 (1937).

(17) O. Piloty and P. Hirsch, *Ann.*, **395**, 69 (1913).

methylpyrazine and tetramethylpyrrole, we treated the fraction in ether with an ether solution of picric acid. The picrate of 2,3,5,6-tetramethylpyrazine separated readily, m.p. 192–193°, but no pure derivatives could be obtained from the lower melting fractions.

Reaction of Propionoin with Alanine.—A mixture of 4.0 g. of propionoin (b.p. 76–78° (40 mm.)) and 3.0 g. of DL-alanine was heated under nitrogen for an hour at 160°. A reflux condenser was used but with occasional release of water vapor. The orange product was filtered from 1.75 g. of unused alanine and diluted with 20 ml. of pentane. On cooling to 0°, 0.25 g. of colorless crystals separated (m.p. 158–160°) which were soluble in water. After crystallization from 95% alcohol, platelet clusters were obtained, m.p. 165–166°. This substance was N-(1-propionylpropyl)-alanine.

Anal. Calcd. for $C_9H_{17}NO_3$: C, 57.7; H, 9.15; N, 7.48. Found: C, 58.2; H, 9.31; N, 7.59.

The oily residue from the pentane extract was taken up in 5 ml. of butyl phthalate and distilled; yield 1.5 g., collected at 150–170° (5 mm.). It possessed a green-blue fluorescence and gave an oily, ether-insoluble picric acid derivative, but was not identified.

Reaction of Acetol with Alanine.—A mixture of 25 g. of acetol (prepared from its acetate by transesterification with methanol[18]) and 30 g. of powdered, ammonia-free DL-alanine was heated (N_2 atm.) at a bath temperature of 120° for 1 hr. under a reflux condenser. The mixture soon turned yellow and in less than 30 minutes became dark brown. Distillation of the contents (to a bath temp. of 150°) yielded about 5 ml. of aqueous material which contained some 2,5-dimethylpyrazine as judged by this evidence: (1) when treated with mercuric chloride in dilute acetic acid, it yielded crystals decomposing[19] without melting above 210°; (2) it yielded a picrate,[20] m.p. 156–157° after crystallization from methanol. *Anal.* Calcd. for $C_{12}H_{11}N_5O_7$: N, 20.77. Found: N, 20.96.

The brown residue was dissolved in 200 ml. of water, filtered from a small amount of undissolved material, mixed with 200 ml. of glacial acetic acid and then treated with a solution of 50 g. of mercuric chloride in 1500 ml. of water. After 3–4 days the 21 g. of precipitate was separated, suspended in water and treated with hydrogen sulfide. After filtration and evaporation of the filtrate 2.2 g. of brown residue was obtained. The absorption spectrum of this material (Fig. 1) was taken: 3.2 mg. in 100 ml. of water. Comparable spectra were obtained by use of 3.3 mg. in 100 ml. of 95% ethanol or 3.2 mg. plus 2.0 mg. of sodium bicarbonate in 100 ml. of water, heated to boiling to expel carbon dioxide.

Non-reaction of Acetol with 2,5-Dimethylpyrazine.—A mixture of 0.4 g. of acetol and 0.5 g. of 2,5-dimethylpyrazine[21] was heated at 120° for 30 minutes in nitrogen. A light yellow color resulted. A similar mixture, heated in air instead of nitrogen, turned a trifle deeper yellow color but did not brown. If 0.1 g. of acetic acid was present, or if pure dimethylpyrazine was heated without acetol, the same yellow color was formed.

Reaction of Acetol with 2,4-Dimethylpyrrole.—A mixture of 0.4 g. of acetol and 0.5 g. of 2,4-dimethylpyrrole[22] (CO_2 atm.) slowly turned red at room temperature. After holding it for 30 minutes at 100° the mass became dark brown. (In a comparable run using tenfold quantities, droplets of water were visibly formed.) The cooled residue was triturated thrice with water yielding a yellow aqueous solution, after which the residue was mixed with 25 ml. of water and distilled to remove any unused dimethylpyrrole. The dried residue (0.67 g.) was dissolved in 95% ethanol and the absorption spectrum (Fig. 1) was taken on an aliquot containing 3.0 mg. per 100 ml.

This brown residue was entirely soluble in ether or concd. hydrochloric acid at this stage, but it became insoluble in both solvents after desiccation over phosphoric anhydride for one week at 1 mm. pressure.

Acetol and Glycine.—A mixture of 4.6 g. of glycine (free of ammonia) and 7.4 g. of freshly prepared acetol was heated under reflux (N_2 atm.) for 20 min. The dark brown mass was cooled, ether extracted (with no more than a trace dissolving), dissolved in 25 ml. of water and filtered from a trace that did not dissolve. To the filtrate was added 50 ml. of glacial acetic acid and then a solution of 15 g. of mercuric chloride in 400 ml. of water. A brown precipitate formed at once, but it gradually increased in quantity. After 6 days it was collected (12 g.), suspended in water and treated with an excess of hydrogen sulfide. After removal of the mercuric sulfide, the filtrate was evaporated; yield 1.3 g. of amorphous, mercury-free, brown precipitate. A solution of this material in acetic acid rapidly changed in color from brown to yellow when reduced by zinc, but on standing the brown color returned.

Three-tenths gram of this amorphous brown product was added to 2 g. of fused potassium hydroxide. There was considerable frothing. The distillate included water and an oil. It caused an acidified (HCl) pine splinter to turn red. It gave a precipitate, m.p. 130°, with mercuric chloride and gave no precipitate with potassium triiodide. These tests point to 2-methylfuran; 5-methyl-2-furylmercuric chloride,[23] m.p. 132, 134°. The -HgCl derivative of pyrrole melts instead at 143°, and pyrrole is known to yield tetraiodopyrrole on reaction with potassium triiodide.

Acetol and β-Phenylalanine.—Five grams of freshly distilled acetol and 4.5 g. of DL-β-phenylalanine was heated under carbon dioxide at 120° for 1 hr. Volatile material was then distilled off, and the brown residue was extracted with ether (yellow-brown solution, but a negligible amount of solute) and water. The residue was then dissolved in 40 ml. of acetic acid and was precipitated with 120 ml. of water, it having been determined previously that phenylalanine is not precipitated under these conditions; yield of brown precipitate, 3.1 g. after drying.

An indication of the amount of phenylalanine residues in the product was obtained by oxidizing 0.453 g. of it to 0.131 g. of benzoic acid, m.p. 119–121°, using potassium permanganate. Under comparable conditions, 0.434 g. of β-phenylalanine yielded 0.246 g. (76% yield) of benzoic acid, m.p. 119–121°. One may assume that the 0.453 g. of product gave rise to 0.175 g. of benzoic acid (since 0.131 g. would represent only three-fourths of it), from which it may be calculated that the ratio of acetol residues to phenylalanine residues in the polymer was 4:1.

Phenacyl Alcohol and Alanine.—Heating of a mixture of 1.36 g. of phenacyl alcohol (m.p. 82–83°) and 0.89 g. of DL-alanine at 120–140° for 15 min. caused strong browning and evolution of carbon dioxide. Part of the mass dissolved in 10 ml. of boiling absolute ethanol. The remainder (0.37 g.) appeared to be 2,5-diphenylpyrazine[24] as crystalline needles melting at 195–201°.

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(18) E. Urion, *Ann. chim.*, [11] **1**, 78 (1934).

(19) H. Fischer and H. Orth, "Die Chemie des Pyrrole," Vol. I, Leipzig, 1934, p. 69.

(20) C. Stoehr, *J. prakt. Chem.*, [2] **47**, 461 (1893), reported 157°.

(21) S. Gabriel and G. Pinkus, *Ber.*, **26**, 2205 (1893).

(22) H. Fischer and R. Müller, *Z. physiol. Chem.*, **148**, 155 (1925).

(23) H. Gilman and collaborators, THIS JOURNAL, **55**, 403, 3302 (1933).

(24) W. Staedel and L. Rügheimer, *Ber.*, **9**, 563 (1876), report the m.p. as 196°.