I, alanine in group II and phenylalanine (which goes into CCl_4 from acid) in group III.

Interesting qualitative demonstrations can be prepared by radio autographing the chromatograms in a sandwich made of two sensitive films, a suitable filter and the chromatogram. The filter is chosen to eliminate S-35 radiation but permit most I-131 radiation to pass through. Any band registered only on the unfiltered side demonstrates the absence of I-131 in it and therefore the absence of the amino acid in question from the sample. A pair of radioautographs showing the absence of hydroxyproline is shown in Fig. 1. The same pair shows the presence of serine and threonine.

Acknowledgements.—The I-131 and S-35 used in this work were supplied by the Oak Ridge National Laboratories on allocation from the Atomic Energy Commission. We are indebted to Dr. Heinrich Rinderknecht for aid in the early stages of this work.

Summary

A method is described for the estimation of glutamic acid, aspartic acid, hydroxyproline, serine and threonine in 0.2–1.0 mg. of hydrolyzed protein. Paper chromatography is applied to the separation of the *p*-iodobenzenesulfonyl derivatives formed from the protein hydrolysate products with a reagent containing I-131. The recovery is measured by the use of indicators consisting of known *p*-iodobenzenesulfonyl derivatives containing S-35 which are added in known amounts immediately after forming the I-131 derivatives of the amino acids. Radioautograms are used to locate the bands and constancy of I-131 to S-35 ratios in successive portions of the bands provides a test of the validity of the analysis.

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[CONTRIBUTION FROM THE RESEARCH DEPARTMENT OF EATON LABORATORIES, INC.]

Furans. II. Ultraviolet Absorption and Molecular Structure

BY ROBERT F. RAFFAUF

In recent years absorption spectra have assumed an increasing importance as an approach to the problems of structural organic chemistry. Although a number of observations on the spectrochemistry of furans have appeared in the literature, much remains to be learned concerning the relationship between the absorption characteristics and the molecular structure of these compounds. The present work was undertaken in order to discover any such general relationships in a series of compounds useful to a more comprehensive study of the chemical and physiological properties of the nitrofurans which has been in progress in this Laboratory. The results are summarized in Tables I and II.

TABLE I

R = 2-furyl

Compound	M. p., °C.	Max. mμ	ε[a]
R—H	...	<220	...
R—CH_2OH	...	<220	...
R—CHO	B. p. 160	230	3500
		275	13750
R—$COCH_3$	30–31	225	3000
		275	13900
R—$COOC_2H_5$	34–35	255	13250
R—CH=$NNHCONH_2$	197–198	290	25000
Furan 2,5-dialdehyde	109–110	290	16900
5-Bromofurfuralsemicarbazone[b]	185 d.	300	27500
5-Methylfurfuralsemicarbazone	195–197	300	24850

[a] In distilled water. [b] Calcd. for $C_6H_6BrN_3O_2$: C, 31.0; H, 2.58. Found: C, 31.3; H, 2.72. *Cf.* Scheibler, *J. prakt. Chem.*, **136**, 232 (1933).

TABLE II

R = 5-NITRO-2-FURYL

Compound	M. p., °C.	Max. mμ	ε[a]
R—H[f]	26–28	225	3400
		315	8100
R—CH_2OH[g]	B. p. 145 (5 mm.)	230	3750
		320	10350
R—CHO[h]	34–37	225	8250
		310	11600
R—$COCH_3$[i]	76–77	225	10200
		310	11500
R—$COOC_2H_5$[j]	102–104	<200	...
		305	10900
R—NO_2[k]	101.5–102	230	7500
		310	11500
R—CH=$NNHCONH_2$[l]	238 dec.	260	13200
		375	15800
R—C(CH_3)=$NNHCONH_2$[i]	248–250 dec.	260	13250
		375	14000
R—CH=NN(CH_3)$COHN_2$[b,m]	213–214	265	13600
		385	16100
R—CH=NNHCONH—CH_3[c,m]	201–202 dec.	265	12600
		380	15700
R—C(CH_3)=NN(CH_3)$CONH_2$[e,m]	187–190 dec.	225	12100
		320	9250
		360	8100

[a] In distilled water. [b] Calcd. for $C_7H_8N_4O_4$: C, 39.6; H, 3.79. Found: C, 39.8; H, 3.63. [c] Calcd. for $C_7H_8N_4O_4$: C, 39.6; H, 3.79. Found: C, 39.9; H, 3.51. [e] Calcd. for $C_8H_{10}N_4O_4$: C, 42.5; H, 4.68. Found: C, 42.9; H, 4.43. [f] Marquis, *Comp. rend.*, **132**, 141 (1901); Marquis, *Ann. chim. phys.*, [8] **4**, 216 (1905). [g] Gilman and Wright, THIS JOURNAL, **53**, 1924 (1931). [h] Gilman and Wright, *ibid*, **52**, 2550, 2552, 4165 (1930); Gilman and Wright, *Rec. trav. chim.*, **50**, 834 (1931). [i] Rinkes, *Rec. trav. chim.*, **51**, 349 (1932). [j] Marquis, *Compt. rend.*, **135**, 506 (1902). Freure and Johnson, THIS JOURNAL, **53**, 1142 (1931). [k] Hill and White, *Am. Chem. J.*, **27**, 193 (1902). [l] U. S. Patent **2,416,234**. [m] These laboratories.

Discussion

Previous work has indicated that although the diene structure of furan and its simple derivatives does not absorb in the near ultraviolet,[1] an extension of the system to include a group capable of conjugation through the 2- or 3-position of the ring results in well-defined absorption above 200 mμ.[2-6] The data which we have recorded for a number of 2-furyl derivatives are in agreement with this principle (Table I). Further, we are able to demonstrate that if this conjugation is supplied through an α-nitro group, the spectrum consists of two absorption bands, one of which falls quite regularly above 300 mμ (Table II).

The data for the aldehydes and ketones and their derivatives listed in Tables I and II are in general agreement with those expected for α,β-unsaturated carbonyl compounds.[7] There are, however, certain anomalous results to be noted in the series. The conjugation and bathochromic effects noted, but not accounted for, by Evans and Gillam in their series of semicarbazones are also to be observed in the series listed in Table II. In addition, it is of particular interest to note the unexpected "washing out" of a portion of the spectrum which results when a methyl group is substituted for hydrogen in the semicarbazone side-chain of the 5-nitrofuryl methyl ketone derivative (Fig. 1). Further, the similarity in the curves for all of the simple nitrofurans suggests that the α-nitrofuryl chromophore contributes to the absorption characteristics of these compounds to the exclusion of the substituent in the α′-position except insofar as the intensity of absorption may reflect structural differences in that position.

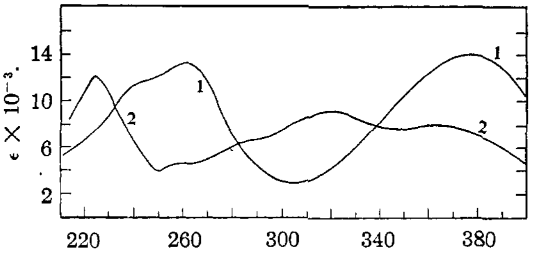
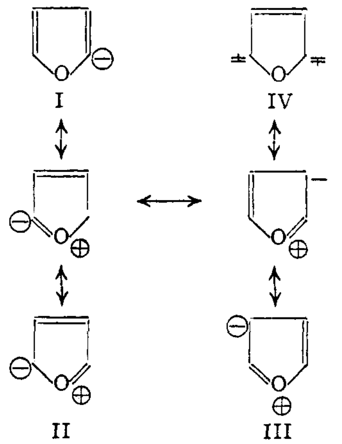
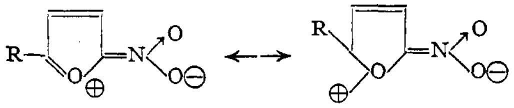


Fig. 1.—Ultraviolet absorption spectra: (1) 5-nitrofuryl methyl ketone semicarbazone; (2) 5-nitrofuryl methyl ketone 2-methylsemicarbazone; determinations in water, n = 10.0 and 10.1 mg./l., respectively.

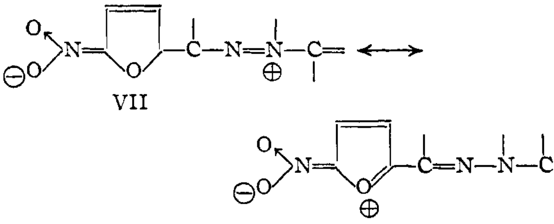
A qualitative approach to an understanding of these trends may be made on the basis of known resonance concepts. Of the principal structures contributing to the resonance hybrid of furan itself probably II is of the greatest importance.[8] If we assume that the introduction of a highly polar nitro group in the α-position of the ring stabilizes structures of type IV, the excited state of nitrofuran molecules would include a relatively large contribution of structures of type VI. On the basis of such structures, the ultraviolet absorption curves for a series of nitrofurans would be expected to be similar to that for nitrofuran itself and to differ from each other only insofar as they reflect the ability of the group R to donate electrons to the nitrofuryl system.[9]





V VI

Extending this concept to the resonance hybrid of the semicarbazones, it will be seen that an increase of the contribution of structures such as VII makes available an extended conjugated system to account for the shift of the absorption maxima of



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[1] Hughes and Johnson, THIS JOURNAL, 53, 737 (1931).

[2] Sorensen, *Ann.*, 546, 57 (1940).

[3] Hoffman, THIS JOURNAL, 66, 51 (1944).

[4] Blout and Gofstein, *ibid.*, 67, 13 (1945).

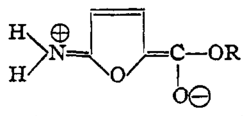
[5] Scallet and Gardner, *ibid.*, 67, 1934 (1945).

[6] A. W. Johnson, *J. Chem. Soc.*, 895 (1946).

[7] Evans and Gillam, *ibid.*, 565 (1943).

[8] Wheland, "The Theory of Resonance," John Wiley and Sons, Inc., New York, N. Y., 1944.

[9] In this connection we have observed that the spectra of methyl 5-aminofuroate ($\lambda_{max.}$ 310, $\epsilon_{max.}$ *ca.* 12,400), and its acetyl derivative ($\lambda_{max.}$ 290, $\epsilon_{max.}$ *ca.* 16,500) are very similar to that of ethyl 5-nitrofuroate. For these compounds analogous structures of the type



may be written. On the other hand, 2-bromo-5-nitrofuran exhibits only one band of greatly weakened intensity (ϵ = *ca.* 3900) at 330 mμ.

these derivatives several millimicrons toward the visible.[10] This behavior is less to be expected from any significant contribution of structures of type VIII.

A Fisher–Hirschfelder model of 5-nitro-2-furyl methyl ketone 2-methylsemicarbazone demonstrates a restricted rotation about the =N—N— bond, induced by the close proximity of the two methyl groups, which is not obtained when either of these groups is replaced by hydrogen. This steric effect, then, reduces the contribution of structures of type VII, increases the probability of type VIII, thus shortening the conjugated system and reducing the spectrum to one more closely allied to nitrofuran itself. Such a concept seems compatible with recently observed effects on spectra due to the steric inhibition of resonance.[11]

On the basis of such an hypothesis one might anticipate that a comparison between the semicarbazone and 2-methylsemicarbazone of a strictly aromatic ketone would demonstrate the same effect. That such is the case may be seen from the data which we obtained for those derivatives of β-acetylnaphthalene (Table III), and their absorption spectra shown in Fig. 2.

TABLE III

Compound	M. p., °C.	Max., mμ	ε[a]
β-Acetyl-naphthalene semicarbazone	235	235	26400
		260	27500
		295	22800
β-Acetyl-naphthalene 2-methyl-semicarbazone[b]	172	245	41700
		285	9950

[a] In 50% ethanol. [b] Calcd. for $C_{14}H_{15}N_3O$: C, 69.7; H, 6.62; N, 17.4. Found: C, 69.9; H, 6.09; N, 17.4.

It is to be expected that an extension of this study in the direction suggested by these data would be productive of a more thorough understanding of the relationship between the absorption spectra and the molecular structure of the furans. No such study is contemplated in this Laboratory at the present time; the application of these data to the identification of the nitrofurans in biological systems will be published elsewhere.[12]

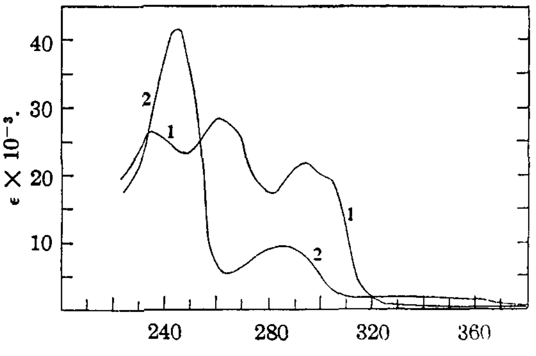


Fig. 2.—The ultraviolet absorption spectra: (1) β-acetylnaphthalene semicarbazone; (2) β-acetylnaphthalene 2-methylsemicarbazone; determinations in 50% ethanol, c = $^1/_{40}$ millimolar.

The compounds described in this report were prepared and purified by methods already well known to the literature. The analytical data for those described for the first time are recorded in the footnotes to Tables II and III. The spectral data were taken in the region 220–400 mμ on aqueous solutions of appropriate concentration (approximately 10 mg./l.) with the exception of the acetylnaphthalene derivatives which were dissolved in 50% ethanol. The instrument used was the Beckman model D quartz spectrophotometer with a hydrogen discharge source.

Acknowledgment.—The author wishes to thank Dr. R. T. Arnold of the School of Chemistry, University of Minnesota, for his helpful suggestions and continued interest during the course of this work.

Summary

Ultraviolet absorption data have been presented for a number of furan and nitrofuran derivatives, and possible relationships between the absorption characteristics and the molecular structure of these compounds have been presented.

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[10] Similar structures have been considered contributory to the resonance hybrid of the aldehyde and ketone 2,4-dinitrophenylhydrazones; *cf.* Roberts and Green, THIS JOURNAL, **68**, 214 (1946).

[11] Reagan and Brown, *ibid.*, **69**, 1032 (1947).

[12] It is possible that the "washing out" of a portion of the spectrum noted with a methyl group is introduced into certain semicarbazones might be due to tautomerism rather than to a damping of resonance. Some evidence in support of that view has been found in the case of analogous furfural derivatives, such as the 2,4-dinitrophenylhydrazone (*cf.* Bredereck, *Ber.*, **65**, 1833 (1932)).

[13] Original manuscript received December 30, 1948.