with standard Beckman *p*H meters. Glass and calomel electrodes were used.

The solutions were prepared as described above and *p*H was determined at ambient temperature. The *p*H values were converted to hydrogen ion concentrations with no correction being applied.

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The Infrared Spectra of N-Substituted Trifluoroacetamides

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Several hitherto unreported N-substituted amides have been prepared and their infrared spectra recorded. Of these compounds, the trifluoroacetamides, exhibit the so-called amide I and amide II[2] absorption bands at 5.8–5.9 and 6.35–6.45 μ, respectively. On the other hand, representative formamides and acetamides (Table I) exhibit these amide bands at 6.0–6.2 and 6.4–6.6 μ, respectively.

TABLE I

AMIDE ABSORPTION BANDS (IN μ) OF SOME TRIFLUOROACETAMIDES, FORMAMIDES AND ACETAMIDES

N Substituent(s)	Trifluoroacetamides Amide I	Trifluoroacetamides Amide II	Formamides Amide I	Formamides Amide II	Acetamides Amide I	Acetamides Amide II
Methyl	..	..	5.98[a]	6.47[a]	...	...
Ethyl	..	..	...	...	6.08[a]	6.42[a]
Isopropyl	5.88	6.38	...	...	...	...
n-Butyl	5.82	6.37	...	...	...	...
Phenyl	5.89	6.43	6.00[a]	6.45[a]	6.01[a]	6.46[a]
Benzyl	5.86	6.42	6.11[a]	6.51[a]	...	...
Diethyl	5.89	[b]	5.98	[b]	6.08	[b]
Di-*n*-propyl	..	..	5.95	[b]	6.06	[b]
Di-isopropyl	5.91	[b]	5.98	[b]	...	...
Di-*n*-butyl	5.88	[b]	5.96	[b]	6.06	[b]
Di-isobutyl	5.90	[b]	...	...	6.05	[b]

[a] These data were obtained from H. M. Randall, *et al.*, reference 2. [b] Amide II band is not present in N,N-disubstituted amides.

This shift to lower wave lengths of the absorptions attributable to the amide grouping is an expected effect of the electronegative trifluorosubstitution in as much as the increased ionic character of the F_3C–C bond increases the carbonyl stretching frequency. In addition to the amide group shifts, the trifluoroacetamides show extremely strong absorption between 8 and 9 μ attributable to C–F stretching. In general, the two bands arising from the symmetrical and antisymmetrical CF_3 stretching modes are clearly resolved. The degeneracy of the latter mode is often removed to such an extent that three bands appear in this region. Regardless of the shape of the 8–9 μ absorption band, it has proved very useful when considered in conjunction with the shifted amide I and II bands in quickly distinguishing between trifluoroacetamides and other types of amides.

(1) University of Toronto, Toronto, Ontario, Canada.

(2) H. M. Randall, R. G. Fowler, N. Fuson and J. R. Dangl, "Infrared Determination of Organic Structures," D. Van Nostrand Co., Inc., New York, N. Y., 1949, p. 11.

Experimental[3]

The infrared data were obtained by means of a Perkin-Elmer model 21 infrared spectrophotometer, using sodium chloride prisms and cells. All liquids were run as pure liquids and the solids as Nujol mulls or melts.

The benzyl and phenyl substituted trifluoroacetamides were prepared according to Bourne, *et al.*[4] The alkyl trifluoroacetamides were all prepared in the same manner as described for diethyl trifluoroacetamide.

N,N-Diethyltrifluoroacetamide.—A solution of 14.6 g. (0.2 mole) diethylamine in 100 cc. of ether was cooled to −10° and 14 cc. (0.1 mole) of trifluoroacetic anhydride was added at a rate such that the reaction temperature remained at −5 to −10°. After the addition was complete, the mixture was warmed to room temperature and washed with water until the wash was neutral to litmus, dried over Na_2SO_4, and distilled at reduced pressure to yield 11.7 g. (69%) of diethyltrifluoroacetamide boiling at 65–67° (24 mm.) with $n^{24}D$ 1.3782.

Anal. Calcd. for $C_6H_{10}ONF_3$: C, 42.59; H, 5.97; N, 8.28. Found: C, 42.0; H, 6.12; N, 8.21.

N-Isopropyltrifluoroacetamide: b.p., 71–72° (20.5 mm.), m.p. 51.0–52.3°. This compound may be purified either by recrystallization from hexane or by vacuum sublimation. *Anal.* Calcd. for $C_5H_8NOF_3$: C, 38.71; H, 5.20; N, 9.03. Found: C, 39.07; H, 4.73; N, 9.27.

N-*n*-Butyltrifluoroacetamide: b.p., 100–101° (28.5 mm.), $n^{25}D$ 1.3805. *Anal.* Calcd. for $C_6H_{10}NOF_3$: C, 42.60; H, 5.96; N, 8.28. Found: C, 42.8; H, 6.22; N, 8.12.

N,N-Di-isopropyltrifluoroacetamide: m.p. 52–52.5°. *Anal.* Calcd. for $C_8H_{14}ONF_3$: C, 48.72; H, 7.17; N, 7.10. Found: C, 48.7; H, 7.58; N, 7.38.

N,N-Di-*n*-butyltrifluoroacetamide: b.p. 106° (16.0 mm.), $n^{25}D$ 1.3997. *Anal.* Calcd. for $C_{10}H_{18}NOF_3$: C, 53.32; H, 8.06; N, 6.22. Found: C, 53.3; H, 8.59; N, 5.94.

N,N-Di-*i*-butyltrifluoroacetamide: b.p. 89.0–89.5° (13.4 mm.), $n^{25}D$ 1.4017. *Anal.* Calcd. for $C_{10}H_{18}NOF_3$: C, 53.32; H, 8.06; N, 6.22. Found: C, 52.9; H, 8.47; N, 6.45.

N,N-Diethylacetamide was prepared by the method of Pictet,[5] b.p. 179–181° (700 mm.), 88.5–91.0° (31.0 mm.), $n^{25}D$ 1.4333.

Di-*n*-propylacetamide was prepared by the method of Chancel[6]; b.p. 94.5° (12.0 mm.), $n^{25}D$ 1.4411.

N,N-Di-*n*-butylacetamide was prepared by the procedure used for diisobutylacetamide; its boiling point (238° (705 mm.), 116.5° (8.0 mm.)) checked that reported by Sowa and Nieuwland[7]; $n^{25}D$ 1.4451.

N,N-Di-isobutylacetamide.—To 240 cc. (2.3 moles) of purified acetic anhydride was added 106 cc. (0.82 mole) of distilled di-isobutylamine, the temperature being allowed to rise to 110°. This solution was distilled at atmospheric pressure to remove 170 cc. of acid and excess anhydride, then at 9.3 mm. to yield 75.3 g. (72%) of amide boiling at 99–102°. A second fractionation through a 12-inch Vigreux gave a product b.p. 102.5–103.0° (9.8 mm.), $n^{25}D$ 1.4434.

Anal. Calcd. for $C_{10}H_{21}NO$: C, 70.09; H, 12.35; N, 8.22. Found: C, 70.6; H, 12.6; N, 7.84.

This compound was previously reported[8] to be a solid, melting at 74°. Our compound could not be caused to crystallize. Since the product of Chute, *et al.*, resulted from a nitration reaction, it probably was di-isobutylnitramine; the latter compound was prepared[9] and found to melt at 81–82°.

Diethylformamide was made by the method of Ott[10]; b.p. 69° (15.0 mm.), $n^{25}D$ 1.4296.

Di-isopropylformamide.—To 46.0 g. (1.0 mole) of 98–100% formic acid in 200 cc. of *m*-xylene was added with cooling 101 g. (1.0 mole) of distilled di-isopropylamine, while the temperature was maintained below 60°. The resulting two-phase liquid was refluxed 48 hours and water continuously removed in a side-arm trap. The solution, now homogeneous, was distilled at atmospheric pressure

(3) All temperatures are uncorrected.

(4) E. J. Bourne, S. H. Henry, C. E. M. Tatlow and J. C. Tatlow, *J. Chem. Soc.*, 1041 (1952).

(5) A. Pictet, *Ber.*, **23**, 3013 (1890).

(6) M. F. Chancel, *Bull. soc. chim.*, [3] **11**, 935 (1894).

(7) F. J. Sowa and J. A. Nieuwland, THIS JOURNAL, **59**, 1202 (1937).

(8) W. J. Chute, *et al.*, *Can. J. Research*, **26B**, 114 (1948).

(9) J. H. Robson, unpublished work.

(10) E. Ott, G. Dittus and H. Meissenburger, *Ber.*, **76B**, 84 (1943).

through an 18-inch Vigreux until the pot temperature rose to 170°, then at reduced pressure to give a fraction, b.p. 93–95° (22.1 mm.). A second fractionation gave 54.0 g. (42%) of amide, b.p. 93–93.8° (23.5 mm.), m.p. 11.6°, $n^{20}D$ 1.4371.

Anal. Calcd. for $C_7H_{15}NO$: C, 65.07; H, 11.70; N, 10.84. Found: C, 64.9; H, 11.7; N, 10.8.

Di-*n*-propylformamide was prepared as previously described[11]; b.p. 206–207° (715 mm.), $n^{25}D$ 1.4384. This amide could not be crystallized at −70°.

Anal. Calcd. for $C_7H_{15}NO$: C, 65.07; H, 11.70; N, 10.84. Found: C, 65.33; H, 11.80; N, 10.57.

(11) J. H. Robson, submitted to THIS JOURNAL for publication.

Di-*n*-butylformamide was prepared by the method of Massie,[12] b.p. 101° (7.8 mm.), $n^{20}D$ 1.4400.

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(12) S. P. Massie, *Iowa State Coll. J. Sci.*, **21**, 41 (1946).

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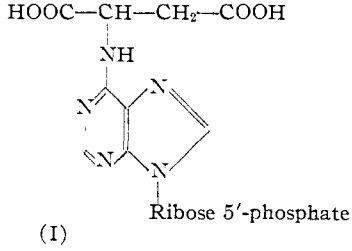
COMMUNICATIONS TO THE EDITOR

ENZYMATIC SYNTHESIS OF ADENYLOSUCCINIC ACID[1]

Sir:

From yeast autolysates a protein fraction has been prepared by ammonium sulfate precipitation and differential heat inactivation which is free of fumarase and catalyzes the reaction

(1) Fumaric acid + Adenosine 5′ phosphate ⇌



(I)

The product of this reaction, tentatively assigned the structure I, [6-(succinylamino)-9-(ribofuranosyl 5′-phosphate)-purine] and the trivial name adenylosuccinic acid (AMP-S), has been isolated by ion exchange chromatography employing the Dowex-1, 2% cross linked resin in the chloride form and elution with a solution 0.02 *N* with respect to HCl and NH_4Cl. A solid amorphous preparation of the compound has been obtained by alcohol precipitation which is 92% pure on spectrophotometric analysis (E_M 267 mμ in 0.1 *N* HCl = 16.9 × 10^3 based on 1 mole of phosphorus), shows only one component on ion exchange and paper chromatography and whose elementary composition is in agreement with a mono-ammonium salt.

The following evidence supports the structure proposed: the organic phosphorus of the compound is quantitatively hydrolyzed to inorganic phosphate by bull semen 5′-nucleotidase[2] which is free of diesterase. Ribose and phosphate occur in the compound in equimolar proportion. The ultraviolet absorption spectrum of I exhibits a maximum at 267 mμ in acid (Fig. 1), a finding which also has been reported by Mason for 6-(methylamino)-purine.[3] Fumaric acid labeled with C^{14} in the carboxyl groups is incorporated enzymatically into I without dilution of relative molar specific activity. Carboxyl C^{14} labeled I is degraded by yeast enzyme free of fumarase at *p*H 7.0 to yield fumaric acid (90% radioactivity recovered) and AMP (equimolar with starting AMP-S), the products being determined by ion exchange and paper chromatography. Although titration data for I do not clearly demonstrate the carboxyl groups in the presence of the nucleotide phosphoryl group, ion exchange analysis indicates that I is more acidic than ADP, a finding in agreement with the structure proposed. That the amino group of adenine is the point of union with the succinyl residue is indicated by the spectral evidence cited and by the finding that muscle adenylic deaminase[4] does not attack I until yeast enzyme has cleaved the compound to yield the AMP moiety. The foregoing evidence supports a reaction mechanism for the enzymatic synthesis of I analogous to the synthesis of argininosuccinic acid by "splitting" enzyme.[5] An average equilibrium constant for reaction (1) calculated for

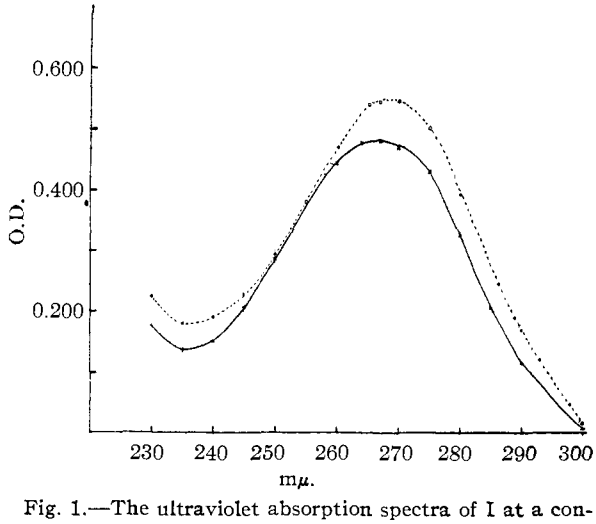


Fig. 1.—The ultraviolet absorption spectra of I at a concentration of 0.0285 μM. per ml. based on phosphorus determination: Solid line, 0.1 *N* HCl; broken line, 0.1 *N* KOH; E_M 267 mμ, *p*H 1.0 = 16.9 × 10^3; E_M 270 mμ, *p*H 12.0 = 19.2 × 10^3.

(1) This work was supported by grants from the U. S. Public Health Service and the Atomic Energy Commission.

(2) L. A. Heppel and R. J. Hilmoe, *J. Biol. Chem.*, **188**, 665 (1951).

(3) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).

(4) H. L. Kalckar, *J. Biol. Chem.*, **167**, 445 (1947).

(5) S. Ratner, "Advances in Enzymology," Vol. 15, p. 319, Interscience Publishers, Inc., New York, N. Y., 1954.