N-(3,5-Dinitro-2-thienyl)amino Acids (I)

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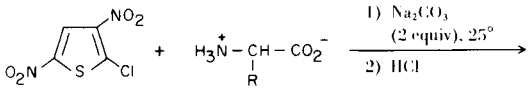
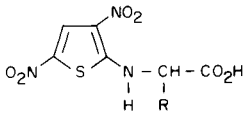
Received September 7, 1971

While examining the nucleophilic substitution of 2-chloro-3,5-dinitrothiophene (I) with enamines (2), it was suggested (3) that the dinitrothienyl group might be an appropriate *N*-label for amino acids. Kinetic studies suggest that I is very labile to nucleophilic displacement; while 1-chloro-2,4-dinitrobenzene reacts approximately 0.001 as fast as 1-fluoro-2,4-dinitrobenzene toward piperidine in alcohol, I is perhaps 0.3-0.4 as reactive as 1-fluoro-2,4-dinitrobenzene (4).





II

(Table I for R)

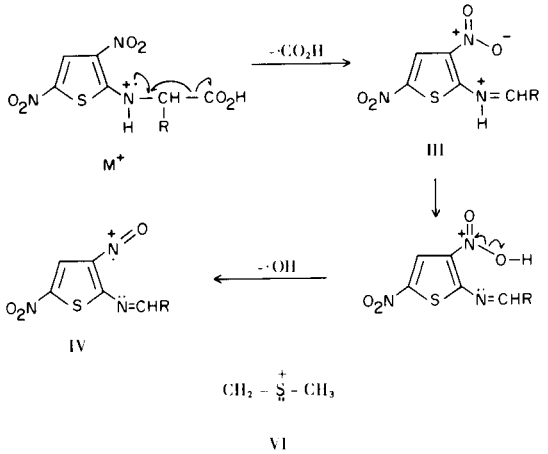
The *N*-(3,5-dinitro-2-thienyl)(DNT)amino acids (II) were synthesized by adding a solution of amino acid in aqueous sodium carbonate to a slurry of I in water, followed by stirring at room temperature. Yields of 30-55% of II were obtained, except for DNT-glycine (II(a), 15%). It was not clear whether the lower yields of DNT-amino acids relative to those reported by Levy and Chung and others (5) for *N*-(dinitrophenyl)(DNP)amino acids was due to loss of I from either hydroxide ion attack to give 3,5-dinitro-2-thienol (unstable) (6) or carboxylate ion attack to give esters (6). Reaction times, yields, analytical and chromatographic data are summarized in Table I.

All of the DNT-amino acids exhibited infrared spectral features very similar to those of the DNP-amino acids (7), namely, absorptions at 3275-3312 (N-H), 1705-1730 (C=O), 1510-1522, 1555-1580 and 1340-1369 (nitro), and 1400-1425 cm^{-1} (carboxylate).

Two features were noted in the nmr spectra of II (Table II). First, the amino proton (singlet) chemical shift was sensitive to traces of moisture. The moisture presumably was introduced in solvent hexadeuteroacetone; this was supported by the presence of an O-H absorption (~ δ 2.8-2.9), as well as non-deuterated solvent absorption (~ δ 2.1), in a solvent blank spectrum (8). Confirmation was observed on addition of a drop of water to the nmr solution of DNT-valine (IIc) and DNT-aspartic acid (IIg); in both the amino proton signal moved upfield and increased in area. Thus, all the amino proton absorptions represent an averaging (in proportion to the relative amounts of II and water) of the nitrogen and hydroxyl proton shifts due to exchange. This had earlier been noted for the amino proton (δ 8.7-9.2 in anhydrous dioxane) in DNP-amino acids where addition of water caused an upfield shift as well as signal broadening (9); we also observed this with DNT-glycine (IIa) in dioxane.

The other nmr feature was several examples of magnetically non-equivalent (MNE) protons, arising due to intrinsic asymmetry of the α-carbon of II and/or slow inversion of the trigonal amino nitrogen (10). In both DNT-valine (IIc) and DNT-leucine (IId) two MNE methyls were present, evidenced by a pair of overlapping doublets

SCHEME I



in each spectrum. Further, the methylene protons in DNT-glycine (IIa) appeared to be non-equivalent; the sharp "triplet" methylene proton signal suggested each α-proton was coupled to the amino proton, leading to a pair of doublets with overlap of the downfield peaks of

TABLE I

Synthetic and Analytical Data for II

	II R-(Abbr.)	Rx. Time (hours)	Yield %	M.p., °C	Molecular Formula	C Analysis Calcd.	C Analysis Found	H Analysis Calcd.	H Analysis Found	R_f (a)	R_f (b)
(a)	H-(Gly)	4	15	219-220 (c)	$C_6H_5N_3O_6S$	29.15	28.83	2.04	1.87	0.13	0.56
(b)	CH_3-(Ala)	19	31	165-167	$C_7H_7N_3O_6S$	32.19	32.08	2.70	2.81	0.28	0.54
(c)	$(CH_3)_2CH$-(Val)	43	55	170.8-171.0	$C_9H_{11}N_3O_6S$	37.37	37.05	3.83	3.75	0.44	0.40
(d)	$(CH_3)_2CH-CH_2$-(Leu)	20	38	140-141	$C_{10}H_{13}N_3O_6S$	39.60	39.57	4.32	4.33	0.47	0.34
(e)	$C_6H_5CH_2$-(Phe)	18.5	48	182-185	$C_{13}H_{11}N_3O_6S$	46.29	46.46	3.29	3.26	0.37	0.23
(f)	$CH_3SCH_2CH_2$-(Met)	21.5	55	114-117	$C_9H_{11}N_3O_6S_2$	33.64	33.85	3.45	3.71	0.33	0.42
(g)	HO_2CCH_2-(Asp)	70.5	39	111-112	$C_8H_7N_3O_8S$	31.48	31.21	2.31	2.35	0.03	0.54
(h)	$HO_2C(CH_2)_2$-(Glu)	76.5	45	191-192	$C_9H_9N_3O_8S$	33.86	33.69	2.84	2.85	0.08	0.60

(a) Benzene-acetic acid (80:20). (b) Formic acid-water (50:50). (c) Lit., m.p. 215-217° [J. M. Tien and I. M. Hunsberger, *J. Org. Chem.*, 25, 2056 (1960)].

TABLE II

NMR (δ) (Acetone-d_6) (a,b,c)

Compd. (R-)	N-H (d)	Cα-H	Other Aliphatic H
IIa (H-)	7.9 bs	4.48 "t" (2.8, 5.6)	
IIb (CH_3-)	7.2 bs	4.58 q (~7) (e)	1.76 d (CH_3) (6.9)
IIc ($(CH_3)_2CH$-)	7.2 s	4.28 q (4.3, 8.4) (f)	2.56 m (Cβ-H); 1.18 d (CH_3) (7.1); 1.14 d (CH_3) (6.9)
IId ($(CH_3)_2CH-CH_2$-)	7.40 s	4.37 m	-($C\beta-H_2$) (g); -(Cγ-H) (g); 1.04 d (CH_3) (5.8); 1.01 d (CH_3) (6.4)
IIe ($C_6H_5-CH_2$-)	7.83 bs	4.71 m	3.50 d (Cβ-H) (4.6); 3.47 d (Cβ-H) (6.8); 7.32 s (Aryl)
IIf ($CH_3-S-CH_2-CH_2$-)	7.9 bs	4.63 m	2.63 bm (width 0.7 ppm) ($-CH_2-CH_2$-); 2.14 s (CH_3)
IIg (HO_2C-CH_2-)	10.06 s	4.93 qn	3.32 d ($C\beta-H_2$) (4.9)
IIh ($HO_2C-CH_2-CH_2$-)	8.95 s	4.55 m	2.50 bm (width 0.5 ppm) ($-CH_2-CH_2$-)

(a) The nmr spectra were determined in 10% w/v solutions. Symbols: s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, m = multiplet, b = broad. J values in parentheses in c.p.s. (b) The carboxyl proton in II appeared as a broad singlet centered in the range of δ 8.9-9.4; in II(g) and II(h) they occurred as separate broad singlets centered at δ 9.5 and 9.7 and at δ 9.2 and 9.4, respectively. Similar results for the carboxyl proton in II were observed in dioxane in the four DNT-amino acids studied. (c) The thienyl-4H proton appeared as a sharp singlet in the range of δ 8.16-8.28. In dioxane this proton occurred at δ 8.32-8.42 in four cases studied. (d) See text for discussion. In dioxane this proton had a shift of δ 7.6-9.3 in four cases. (e) further split? (f) Treated as M of an AMX system. (g) Absorption covered by absorption at δ 2.1 due to non-deuterated solvent.

TABLE III

m/e (Relative Intensity)

II	M^+	$(M-45)^+$	$(M-62)^+$	Others (m/e > 170, relative intensity > 10) (a)
IIa	247 (100)	202 (66)	185 (41)	201 (23), 189 (21), 184 (28), 174 (17), 169 (40)
DNP-Gly	241 (22)	196 (100)	179 (22)	163 (48)
IIb	261 (41)	216 (100)	199 (9)	217 (9), 183 (4)
IIc	289 (41)	244 (100)	227 (14)	243 (10), 211 (19), 209 (10), 196 (24), 184 (16), 174 (10), 170 (18)
IId	303 (23)	258 (81)	241 (21)	259 (10), 242 (10), 225 (29), 224 (13), 215 (18), 211 (12), 210 (16), 202 (10), 199 (22), 198 (10), 185 (13), 184 (23), 183 (100), 178 (32), 177 (11), 170 (25)
IIe	337 (100)	292 (79)	275 (27)	293 (29), 259 (94), 258 (41), 257 (31), 246 (19), 229 (30), 213 (22), 212 (23), 200 (19), 184 (38), 170 (37), 91 (1300)
IIf	321 (3)	276 (10)	259 (5)	275 (15), 243 (35), 242 (34), 228 (100), 215 (27), 198 (15), 197 (45), 196 (48), 195 (20), 182 (25), 170 (22), 61 (410)
IIg	305 (- - -)	Mostly thermal decomp.		215 (40), 189 (86), 178 (100)
IIh	319 (6)	Mostly thermal decomp.		301 (6), 273 (11), 256 (46), 241 (76), 223 (36), 196 (100), 195 (86)
V (b)	152 (71)	- -	- -	135 (12) (M-17), 119 (20) (M-33), 106 (73), 105 (100), 104 (50), 91 (32), 79 (19), 77 (72)

(a) For IIe-IIh, relative intensity > 20. (b) peaks of m/e > 70.

TABLE IV

Hydrolysis Data

II	ϵ (II)	% Decomposition (12 hours) II (this study)	% Decomposition (12 hours) DNP-Amino Acid (a) (109.5°, 5.7*N* HCl)
At 355 mμ, 105°, 5.7*N* HCl			
DNT-Gly	9,150 (b)	74 (59-8 hours)	60 (8 hours)
DNP-Gly (c)	10,600 (b)	49 (41-8 hours) (d)	- -
DNT-Phe	10,150	9	30
DNT-Val	9,100 (b)	5	20
DNT-Met	11,600	26	25
DNT-Asp	11,850	19	40 (24 hours)
DNT-Glu	11,050	11	25
At given mμ, 100°, 6.0*N* HCl			
DNT-Ala	10,600 (358 mμ)	17	20
DNT-Phe	9,460 (360 mμ)	4	30
DNT-Leu	15,000 (b)	2	20

(a) Reference 13, a chromatographic-colorimetric procedure. (b) 350 mμ. (c) At 25°, ϵ, 12,420 (350 mμ); lit. ϵ, 12,840 (360 mμ) at *p*H 1 [L. K. Ramachandran and L. V. S. Sastry, *Biochemistry*, 1, 75 (1962)]. (d) Reference 14, a chromatographic procedure, 70% (12 hours, 105°).

each. No geminal coupling was apparent. Similarly, the β-methylene protons ("triplet") in DNT-Phe (IIe) appeared to be non-equivalent, with coupling to the α-proton. Several other examples (IId, IIf, IIh) of possible MNE protons went unseen due to overlap of absorptions; only in IIg were MNE protons not observed.

The mass spectra of II (Table III) suggested that the major fragmentation pattern for IIa-IId ions was the loss of the carboxyl radical to give III $(M-45)^+$ (Scheme I), followed by loss of hydroxyl radical to give IV $(M-62)^+$.

The latter process was reported earlier for *o*-nitroaniline (11) and was also observed in this laboratory in the mass spectrum of *o*-nitro-*N*-methylaniline (V). Additionally, the $(M-62)^+$ ions from IIa-IId and V exhibited loss of an oxygen atom to give $(M-78)^+$ ions of intensity comparable or exceeding that of the $(M-62)^+$ ions; this loss has been reported earlier for a number of functional groups (11), most recently in nitrones (12). The unique base peak of $(M-120)^+$ for IId suggested an additional fragmentation process unique to IId, perhaps including a McLafferty rearrangement that yields isobutene.

For IIe and IIf the fragmentation pattern described above was less prominent; for IIe, fragmentation to the tropylium ion (m/e 91) was most important, while α-cleavage to yield VI (m/e 61) was predominant for the IIf ion. The DNT-amino acids IIg and IIh underwent thermal decomposition almost entirely (13).

Since strong acid hydrolysis normally follows an *N*-labeling reaction of terminal acids in polypeptides and proteins, the extent of decomposition of DNT-amino acids in 5.7-6.0 *N* hydrochloric acid was studied. The results are given in Table IV. Rough comparison of the percent decomposition of II with that of the corresponding DNP-amino acid (14,15) suggests that, except for glycine and perhaps methionine, the 3,5-dinitro-2-thienyl group may serve as an amino acid *N*-label of stability comparable to the 2,4-dinitrophenyl group.

EXPERIMENTAL

Melting points were obtained on a Thomas Hoover apparatus and are uncorrected. Spectra were obtained on Perkin-Elmer Model 621 (Infrared; potassium bromide discs), Varian Model A-60 (nmr; TMS as internal standard), Hitachi Perkin-Elmer Model RMU-6E (mass; chamber voltage = 80 eV), and Cary Model 14 and Perkin-Elmer Model 202 (ultra-violet) spectrophotometers. Carbon-hydrogen analyses were performed by C. F. Geiger, Ontario, California, and M-H-W Laboratories, Garden City, Michigan.

General Procedure for *N*-(3,5-Dinitro-2-thienyl)amino Acids.

To a measured amount (about 0.007 mole) of I (6) mixed with 25 ml. of water (light excluded) was added dropwise (10 minutes) an aqueous solution containing an equimolar amount of racemic amino acid plus two equivalents of sodium carbonate (three for aspartic and glutamic acids). After stirring the deep red mixture for at least 19 hours, filtration removed unreacted I and the filtrate acidified with hydrochloric acid to Congo Red. The yellow-gold solution was extracted 15 times with 10 ml. portions of diethyl ether; however, DNT-gly, DNT-leu and DNT-glu were extracted by continuous liquid-liquid extraction (diethyl ether). After drying the combined ether extracts over anhydrous magnesium sulfate, rotary evaporation left either yellow-brown crystals or a dark brown oil. All derivatives except DNT-gly (methanol-water) were recrystallized from diethyl ether-petroleum ether (30-60°). See Table I for reaction times, yields, and analytical data.

Two-dimensional Thin-layer Chromatography of DNT-amino Acids.

Two ml. of a solution containing one mg. each of the eight DNT-amino acids dissolved in ten ml. of methanol was spotted on a Baker-flex Polyamide 6 sheet (20 x 20 cm). Solvent systems benzene-acetic acid (80:20) and formic acid-water (50:50) developed by Wang *et al.* (16) were used successively (20 minutes for 10 cm development) to give well-separated, small spots. The R_f values are in Table I.

Determination of Hydrolytic Stability of II.

An appropriate, weighed sample of II was diluted with 5.7 *N* hydrochloric acid, used at 105.0 ± 0.4°, to give a solution about 5×10^{-5} *M* in II; 6.0 *N* acid was used at 100.0 ± 0.1°. Aliquots were periodically withdrawn from the heated solution and the remaining II was determined by ultraviolet spectroscopy in the range of 350-360 mμ; the runs were followed to 12 hours (105°) up to seven days (IId, IIe) at 100°. After analysis of the data *via* a standard least squares program for first-order reactions (IBM-360), the computer data was used to obtain the extinction coefficient of II at the reaction temperature, and the percent decomposition of II; correction for solvent expansion was made. Table IV summarizes the results.

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