X-Ray Contrast Media

Glutaric and Iminodiacetic Acids as Carriers of Iodinated Phenyl Ring

By GAD SHTACHER*

Iodinated derivatives of 3-phenyl-glutaric acid and *N*-benzyl-iminodiacetic acid were prepared. The free acids and their dimethyl esters were screened as potential intravenous and oral cholangiographic agents in cats and compared with presently available agents. The oral agents were present mostly in the intestines showing good X-ray intensity, with little or no visualization of the gall bladder and bile ducts. The X-ray intensity of the intravenous agents was not comparable to that obtained with sodium iodipamide. Attempted triiodination of 2-(3′-aminophenyl)-trimethylenedinitrilo-tetraacetic acid failed, probably due to steric factors.

IN SEARCH FOR improved X-ray contrast agents in intravascular radiography, attention was drawn to the biological properties of polyaminopolycarboxylic acids (1). Ethylenediaminetetraacetic acid (EDTA), the prototype of the synthetic polyaminopolycarboxylic acid group, is not metabolized in mammals, and following its parenteral administration, is very rapidly and almost completely eliminated unaltered through the kidneys. In considering the physicochemical properties of EDTA and structurally related compounds, their chemical stability as well as their high water solubility at physiologic pH are additional essential properties required from intravascular radiopaque media. The chelating property of EDTA has in fact been utilized a few years ago, to study the usefulness of lead-EDTA chelate as an X-ray contrast medium in animals and in man (2). This complex, however, proved to be quite unsafe due to release of free lead ions *in vivo*, thus resulting in heavy metal poisoning. Since the pharmacological as well as toxic effects of EDTA, and in particular its marked nephrotoxicity, have been attributed to its powerful chelating ability, which leads to the removal of essential metal ions, it seemed worthwhile to prepare lower and higher homologs of it, namely, iminodiacetic acid and trimethylenedinitrilo tetraacetic acid, respectively, linked to iodinated phenyl rings. The chelate stability constants of these complexing agents with various metal ions are a thousand to a million times lower than those of EDTA (3), thus practically eliminating the danger of essential metals depletion. On the other hand, however, these compounds retain the characteristic biological and physicochemical properties of polyaminopolycarboxylic acids mentioned above. It was also hoped that a combination of an iodinated nucleus with the amino acid side chains would favor the hepato-biliary elimination of the iodinated compounds, following their intravascular administration. It has been repeatedly shown that presence of iodine atoms in organic compounds markedly enhance their biliary excretion, with a concomitant fall in their renal excretion (4). As part of the reaction sequence leading to the preparation of 2-substituted phenyl-trimethylenedinitrilo-tetraacetic acid, iodinated derivatives of 3-phenyl-glutaric acid were also prepared, since their structure resembles to some extent that of 3-(3′-amino-2′,4′,6′-triiodophenyl)-2-ethylpropionic acid (iopanoic acid), currently used for oral cholecystography. Glutaric acid substituted in the α-position with a 3-amino-2,4,6-triiodobenzyl group was recently synthesized as a potential cholecystographic agent, but was found to be quite toxic (5). It was hoped that symmetrically substituted glutaric acid will show lower toxicity combined with enhanced choleretic property.

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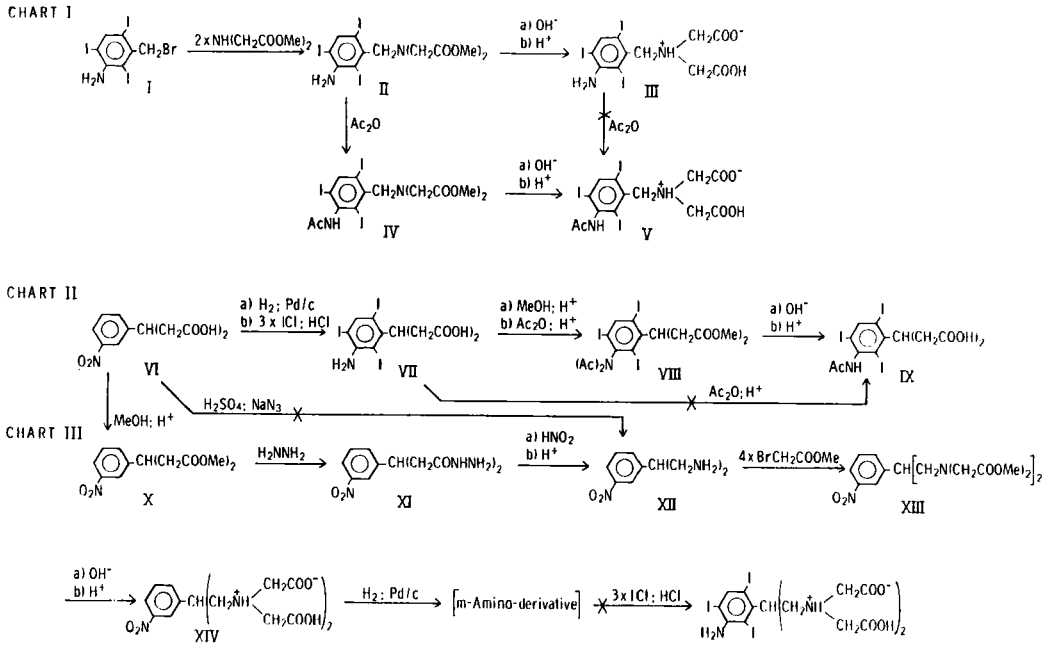
CHEMISTRY

Charts I, II, and III (Scheme I) represent the synthetic approaches to *N*-(3-acetamido-2,4,6-triiodobenzyl)-iminodiacetic acid (V), 3-(3′-acetamido-2′,4′,6′-triiodophenyl)-glutaric acid (IX), and 2-(3′-nitrophenyl)-trimethylenedinitrilo tetraacetic acid (XIV), respectively. 3-Amino-2,4,6-triiodobenzyl bromide (I) was prepared from 3-nitrobenzaldehyde in three steps consisting of catalytic reduction using



Scheme I

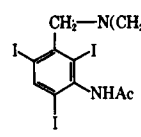
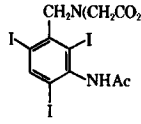
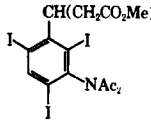
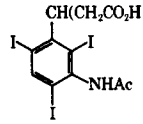
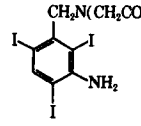
Adams' platinum catalyst (6), iodination with iodine monochloride, followed by treatment with phosphorus tribromide (7). 3-(3′-Nitrophenyl)-glutaric acid (VI), the starting material in Charts II and III (Scheme I) was readily obtained from 3-nitrobenzaldehyde by piperidine-catalyzed condensation of the aldehyde with 2 moles of ethyl acetoacetate, followed by alkaline hydrolysis of the resulting ethyl-3-nitrobenzylidenebis-acetoacetate (8). The most practical approach to the synthesis of 2-(3′-nitrophenyl)-trimethylenedinitrilo tetraacetic acid (XIV), appeared in the alkylation of 2-(3′-nitrophenyl)-trimethylene-diamine (XII) either with chloroacetic acid, or with methyl bromoacetate, followed by saponification of the resulting tetramethyl ester. The nitroaryl diamine (XII) was successfully prepared by the Curtius degradation (9) starting with the corresponding dimethylester (X), and proceeding through the dihydrazide intermediate (XI). Attempts to synthesize the diamine (XII) directly from 3-(3′-nitrophenyl)-glutaric acid by the Schmidt reaction (10) failed. The final step in the reaction sequence of Chart III, namely, triiodination of 2-(3′-aminophenyl)-trimethylenedinitrilo-tetraacetic acid, using either iodine monochloride or potassium iododichloride as iodinating agents, was unsuccessful. Titration of an aliquot sample of the iodination reaction mixture, indicated that only one-third of the theoretical quantity of iodine monochloride had been consumed. It thus appears that steric hindrance exerted by the trimethylenedinitrilo-tetraacetic acid side chain, probably blocked positions 2 and 6 on the benzene ring which were otherwise available for iodine substitution. Direct acetylation of either *N*-(3-amino-2,4,6-triiodobenzyl)-iminodiacetic acid (III), or 3-(3′-amino-2′,4′,6-triiodophenyl)-glutaric acid (VII) to give the corresponding ar-acetamido derivatives, always led to a tarry product, presumably due to the formation of cyclic carboxylic anhydrides which later polymerized. Blocking of the carboxyl groups by methyl ester formation, enabled the acetylation reaction to proceed smoothly in good yield, after which saponification of the acetylated products yielded the desired acetamido-triiodophenyl-carboxylic acids.

PHARMACOLOGY[1]

The acute toxicites of the test compounds are listed in Table I. The test compounds were screened as cholangiographic agents; three of them (IV, VIII, and IX) as oral agents, and three (III, V, and IX) as intravenous agents. Four mongrel cats were used for each compound tested. For intravenous cholangiographic (i.v.c.) screening the cats were fasted 24 hr. prior to dosing. Sixteen hours before the test, 75 ml. of evaporated milk mixed with 25 ml. of tap water was fed to the animals. All animals were dosed with 66 mg. iodine/kg. body weight. For oral cholangiographic screening the same procedure as for the i.v.c. screening was followed. The only variation was the fasting time which was extended to 32 hr., with the evaporated milk fed 6 hr. prior to dosing. Compounds IV and VIII were administered orally at a dose of 60 mg. iodine/kg. body weight. The X-ray intensity of the intravenous agents III, V, and IX was not comparable to that obtained with sodium iodipamide, the reference compound for intravenous cholangiography. The oral agents were present mostly in the intestines of the cats, showing good X-ray intensity. Only one cat treated with Compound IV showed good absorption with excretion of the test compound by way of the biliary system. However, visualization of the bile duct was vague and incomplete.

[1] Private communication. Van Derike, D. R., Valenti, A., and Hoey, G. B., Mallinckrodt Chemical Works, Pharmaceutical Division, St. Louis, Mo.

TABLE I—ACUTE TOXICITY AND DEGREE OF RADIOGRAPHIC VISUALIZATION OF THE GALL BLADDER AND BILE DUCT OF THE VARIOUS CONTRAST MEDIA

Compd. No.	Structure	LD_{50} (g./kg.)[a]	Cholecystography Screening[b]
IV		>10.0[c]	Not absorbed[c]
V[d]		≈6.0[e]	Poorly visualized[e]
VIII		—	No visualization[c]
IX		≈3.0[e]	Poorly visualized[e] No visualization[c]
III[d]		2.4[e]	Poorly visualized[e]

[a] Mice. [b] Cat. [c] Oral administration. [d] Exist in zwitterionic form. [e] Intravenous administration.

EXPERIMENTAL[2]

2,4,6-Triiodo-3-aminobenzyl Bromide (I)—This was prepared in 72% yield by treating the corresponding alcohol with phosphorus tribromide, according to Hebký and Karásek (7). Dioxane was found to be a better solvent for crystallization of (I) than chlorobenzene or tetrahydrofuran, m.p. 164–165° [lit. (7) m.p. 165°].

N-(2,4,6-Triiodo-3-aminobenzyl)-iminodiacetic acid Dimethyl Ester (II)—A mixture of 56.4 g. (0.1 mole) of 2,4,6-triiodo-3-aminobenzyl bromide (I), and 32.2 g. (0.2 mole) of iminodiacetic acid dimethyl ester (11) dissolved in 350 ml. of dry dioxane was stirred and refluxed for 3 hr. The reaction mixture was protected from moisture and CO_2 by soda-lime tube. The hydrobromide salt of iminodiacetic acid dimethyl ester began to separate within a few minutes following refluxing. At the end of the reaction, the reaction mixture was cooled, the hydrobromide salt collected by filtration (22.7 g., 94% yield), and the filtrate was concentrated *in vacuo*. The residue (the crude product) was obtained as a dark brown viscous oil which gradually solidified upon cooling. Two crystallizations of the crude product from methanol, furnished 43.2 g. (67% yield) of an analytically and chromatographically pure substance, as long colorless plates, m.p. 89.5–90.5°.

Anal.—Calcd. for $C_{13}H_{15}I_3N_2O_4$: C, 24.25; H, 2.35; I, 59.10; N, 4.35; mol. wt. 644.0. Found: C, 24.40; H, 2.39; I, 59.35; N, 4.43; mol. wt. 647.5. Infrared absorption spectra (CCl_4) $\lambda_{max.}$: 2.89 μ and 2.97 μ (medium, N—H stretching vibrations), 3.40 μ (medium, aliphatic C—H stretching vibration), 5.70 μ (very strong, C=O stretching vibration of ester), 6.27 μ (strong, probably overlapping of N—H deformation absorption and aromatic ring vibrations), 6.92 μ (strong, aromatic C=C stretching vibration), 8.40 μ (very strong, C—O—C stretching vibration of ester).

N-(2,4,6-Triiodo-3-aminobenzyl)-iminodiacetic Acid (III)—Saponification of the *tert*-imino dimethyl-ester (II) was performed in aqueous methanol, using 2 *N* NaOH in 10% excess. The saponification was completed after 10–15 min. of reflux. The alkaline reaction mixture was cooled, extracted with ether, and acidified carefully with concentrated HCl to pH ~ 1. The precipitated imino acid was filtered, washed with water, and crystallized from a mixture of acetic acid–water. The pure product was obtained as colorless fine needles, m.p. 178–179° dec.

Anal.—Calcd. for $C_{11}H_{11}I_3N_2O_4$: C, 21.45; H, 1.80; I, 61.81; N, 4.55; mol. wt. 615.97. Found, C, 21.21; H, 2.01; I, 62.10; N, 4.48; mol wt.: 618.0.

N-(2,4,6-Triiodo-3-acetamidobenzyl)-iminodiacetic Acid Dimethyl Ester (IV)—*N*-(2,4,6-Triiodo-3-aminobenzyl)-iminodiacetic acid dimethyl ester, 32.2 g. (0.05 mole), was acetylated with a mixture of 70 ml. of acetic anhydride, 70 ml. of glacial acetic acid, and 1 ml. of concentrated H_2SO_4. The reaction mixture was heated in an oil bath at 70° for 3 hr., cooled, and poured into a mixture of ice water. The heavy brown oil which separated was extracted with chloroform. The chloroform after drying over anhydrous $MgSO_4$ was evaporated *in vacuo*, and the residue (the crude product) was obtained as a viscous yellow oil. On TLC (solvent, ethyl acetate), the crude product contained approximately 10–20% of the starting material. The crude product gradually crystallized upon cooling, and after two crystallizations from benzene, 20.5 g. (60% yield) of an analytically and chromatographically pure substance were obtained as colorless tiny plates, m.p. 108–109°.

Anal.—Calcd. for $C_{15}H_{17}I_3N_2O_5$: C, 26.26; H, 2.50; I, 55.50; N, 4.08; mol. wt. 685.06. Found: C, 26.31; H, 2.37; I, 55.80; N, 4.19; mol. wt. 689.5. Infrared absorption spectra $\lambda(CHCl_3)_{max.}$: 2.93 μ (medium, N—H stretching absorption of secondary amide), 3.33 μ (strong, aliphatic, C—H stretching vibrations), 5.73 μ (very strong, C=O stretching vibrations of ester), 5.88 μ (strong, C=O absorption of anilide), 6.94 μ (strong, aromatic C=C stretching vibration), 8.37 μ (very strong C—O—C stretching vibration of ester).

N-(2,4,6-Triiodo-3-acetamidobenzyl)-iminodiacetic Acid (V)—The free amino acid was obtained by saponification of the corresponding dimethyl ester (IV) in aqueous methanol (2 *N* NaOH, 10% excess), at 70° for 5 hr. The alkaline reaction mixture was cooled, extracted with ether, and carefully acidified to pH ~ 1 with concentrated HCl. The brown

[2] Melting points were determined on a Fisher-Johns melting point apparatus. All melting and boiling points are reported as uncorrected values. Infrared spectra (Figs. 1 & 2) were recorded on a Perkin-Elmer Infrared spectrophotometer model 137, calibrated by polystyrene film. Chromatography was carried out on layers of Silica Gel H(E. Merck AG., Germany) at a thickness of 0.25 mm. The compounds were detected by iodine vapor.

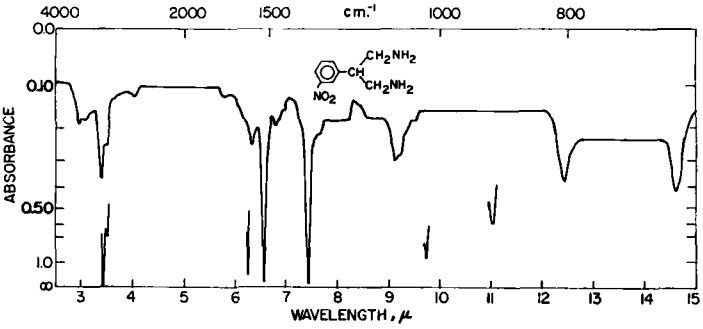


Fig. 1—Infrared absorption spectrum of 2-(3′-nitrophenyl)-trimethylenediamine (XII), 10 mg./ml. in chloroform.

heavy oil which separated was washed several times with water and dried over NaOH pellets *in vacuo*. The resulting solid product was then crystallized from a mixture of acetone–ether to give colorless fine needles, m.p. 128–130° dec. Upon storing, the product slightly decolorized.

Anal.—Calcd. for $C_{13}H_{13}I_3N_2O_5 \cdot 0.5\,H_2O$: C, 23.41; H, 2.11; I, 57.09; N, 4.20; mol. wt. 667.1. Found: C, 23.42; H, 2.16; I, 56.65; N, 4.08; mol. wt. 670.5.

Thin-layer chromatography (MeOH–AcOEt, 9:1), indicated the presence of a trace impurity with a higher R_f value. Attempts to obtain V by direct acetylation of III yielded only tarry material.

3-(3′-Nitrophenyl)-glutaric acid (VI)—This was prepared in 85% yield by a previously reported two-step procedure (8), m.p. 205–207° [lit. (8) m.p. 204–205°].

3-(3′-Amino-2′,4′,6′-triiodophenyl)-glutaric acid (VII)—A suspension of 10 g. of VI (0.04 mole) in 100 ml. of 2 *N* HCl was shaken with hydrogen and 1 g. of 5% Pd/C in a Parr low-pressure hydrogenator. After the theoretical quantity of hydrogen for reduction of the nitro group had been absorbed (*ca.* 2 hr.), the solution was filtered to remove the catalyst and the filtrate was diluted to 1 l. with 0.5 *N* HCl. Iodine monochloride, 23 g. (0.141 mole) in 30 ml. of concentrated HCl was added in one portion to the stirred solution at 55°. Appearance of the product as a heavy yellow to light brown granular precipitate began after about 4 hr. To complete the reaction, heating and stirring were continued for 6 days, a second batch of 23 g. of ICl in 30 ml. of concentrated HCl being added on the third day. After cooling the reaction mixture, the precipitated product was filtered, washed with water, and crystallized from a mixture of methanol–water. Recrystallization from glacial acetic acid yielded 14.2 g. (59% yield) of light cream-colored crystalline product, analytically and chromatographically pure, m.p. 224–226° dec.

Anal.—Calcd. for $C_{11}H_{10}I_3NO_4$: C, 21.98; H, 1.68; I, 63.36; N, 2.33; mol. wt. 600.96. Found: C, 22.05; H, 1.70; I, 63.50; N, 2.25; mol. wt. 597.7.

The product is soluble in methanol, ethanol, and *n*-propanol; it is only slightly soluble in isopropanol and acetone, and insoluble in chloroform, ethyl acetate, benzene, and toluene. Since direct acetylation of VII to yield 3-(3′-acetamido-2′,4′,6′-triiodophenyl)-glutaric acid (IX) failed to give the desired product, IX was obtained by acetylation of the dimethyl ester of 3-(3′-amino-2′,4′,6′-triiodophenyl)-glutaric acid, and subsequent saponification.

3-(3′-Diacetamido-2′,4′,6′,-triiodophenyl)-glutaric Acid Dimethyl Ester (VIII)—A mixture of 12 g. (0.02 mole) of the glutaric acid derivative (VII) in 100 ml. of absolute methanol containing 1 ml. of concentrated H_2SO_4 was gently refluxed for *ca.* 18 hr. The resulting red clear solution was cooled and then poured into 100 ml. of ice water. The dimethyl ester which separated as a heavy red-brown oil was extracted with chloroform. The chloroform extract was consecutively washed with sodium bicarbonate 10% solution and water, and then dried over anhydrous $MgSO_4$. After evaporation of the chloroform under reduced pressure, 10.1 g. (80% yield) of the product was obtained as a very viscous, clear, red-colored oil, which was chromatographically pure (solvent: methanol). The oily product did not crystallize during a long storage period in the cold and was used as such for the acetylation reaction. The dimethyl ester 12.6 g. (0.02 mole) was acetylated with 60 ml. of acetic anhydride containing 0.5 ml. of concentrated H_2SO_4. The reaction mixture was heated in an oil bath at 70° for 18 hr., cooled, and poured into 200 ml. of ice water. The heavy red-brown oil which separated was extracted with chloroform. The chloroform, after being washed with water and dried over anhydrous $MgSO_4$, was evaporated under reduced pressure, and the residue

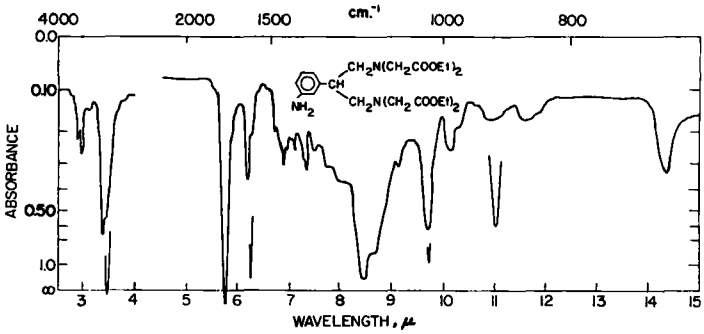


Fig. 2—Infrared absorption spectrum of 2-(3′-aminophenyl)-trimethylenedinitrilo-tetraacetic acid, tetraethyl ester, 10 mg./ml. in carbon tetrachloride.

(the crude product) was obtained as a viscous, clear, red-colored oil which gradually crystallized on storing in an ice box. Two crystallizations of the crude product, one from methanol and one from ether gave 12.1 g. (85% yield) of an analytically and chromatographically pure substance, in the form of colorless long plates, m.p. 102–103°.

Anal.—Calcd. for $C_{17}H_{18}I_3NO_6$: C, 28.63, H, 2.54; I, 53.40; N, 1.96. Found: C, 28.54; H, 2.49; I, 53.25; N, 1.92.

The product is soluble to a varying extent in most organic solvents.

3-(3′-Acetamido-2′,4′,6′-triiodophenyl)-glutaric Acid (IX)—A suspension of 21.4 g. of VIII (0.03 mole) in 60 ml. of 2 *N* NaOH, was stirred and heated to 80° for 24 hr. Complete solution occurred after 2 hr. The alkaline reaction mixture was then cooled, extracted with ether, and acidified with concentrated HCl. A heavy, cream-colored semisolid separated. The aqueous solution was decanted and the viscous precipitate washed twice with distilled water and dried over KOH pellets *in vacuo* at 50° overnight. The resulting cream-colored crude product weighed 18.3 g. and melted in the range of 140–160°. Two crystallizations from glacial acetic acid furnished 13 g. (67% yield) of analytically and chromatographically pure substance, in the form of colorless fine needles, m.p. 233–235° dec. Mixed m.p. with VII (the nonacetylated derivative), 195–205° dec.

Anal.—Calcd. for $C_{13}H_{12}I_3NO_5$: C, 24.28; H, 1.88; I, 59.22; N, 2.18; mol. wt., 643.0. Found: C, 24.28; H, 1.88; I, 59.35; N, 2.16; mol. wt., 645.5.

The product is freely soluble in DMF, only moderately soluble in hot methanol, ethanol, and acetic acid, and insoluble in water and most other organic solvents

3-(3′-Nitrophenyl)-glutaric Acid Dimethylester (X)—A mixture of 25.3 g. (0.1 mole) of VI in 150 ml. of absolute methanol containing 4.5 ml. of concentrated H_2SO_4 was gently refluxed for *ca.* 20 hr. Fifty milliliters of methanol was then distilled off, and the resulting clear dark red solution was cooled in ice water. Within minutes, the entire solution became a thick crystalline paste of the dimethyl ester. The brown-colored crystalline product, after filtration and thorough wash with water, was taken in chloroform. The aqueous filtrate was extracted with chloroform and the combined extracts were consecutively washed with sodium bicarbonate 10% solution and water and then dried over anhydrous calcium chloride. After evaporation of the chloroform under reduced pressure, the residue was distilled in high vacuum. The pure dimethyl ester was obtained as a yellowish clear distillate which crystallized on standing. Yield: 22.8 g. (81%), b.p. 163° (0.07 mm.), m.p. 65–66°. Crystallization from isopropyl ether or methyl-cyclohexane gave colorless needles of the product, without affecting the melting point. The dimethyl ester is soluble in most organic solvents except for ether, isopropyl ether, and methyl-cyclohexane from which it crystallizes on cooling. The NMR spectrum of (X) in $CDCl_3$ (60 Mc., TMS reference standard), showed a doublet centered at δ 2.75 of spacing 7 c.p.s. due to the four protons of the two methylene groups, a singlet at 3.59 due to the overlapping of six protons of the two OCH_3 groups, triplet of triplets centered at 3.80 of spacing 8 c.p.s due to the benzyl proton, and two multiplets each accounting for two aromatic protons centered at 7.38 and 7.96, respectively.

Anal.—Calcd. for $C_{13}H_{15}NO_6$: C, 55.51; H, 5.38; N, 4.98. Found: C, 55.62; H, 5.28; N, 5.03.

3-(3′-Nitrophenyl)-glutaryl Hydrazide (XI)—A solution of 28.1 g. (0.1 mole) of the dimethyl ester (X) in 150 ml. of methanol was added dropwise over a period of 0.5 hr. to a boiling stirred solution of 8 g. (0.25 mole) of hydrazine in 30 ml. of methanol. After completion of the addition, boiling and stirring were continued for another 0.5 hr. "Seeding," or little scratching of the hot solution caused immediate crystallization of the product. After cooling in ice water, the white crystalline paste of the hydrazide was filtered and washed with methanol and ether. After drying in air and at 80°, the hydrazide weighed 26.7 g. (95% yield), and was analytically pure, m.p. 184–186°. Crystallization from water or ethanol did not change the melting point. Since the preparation of hydrazides in the nitroaromatic series sometimes involves complications due to the reducing action of hydrazine, the presence of nitro group in the product (XI) was confirmed by infrared spectroscopy. The infrared spectra of 3-(3′-nitrophenyl)-glutaryl hydrazide contained the expected characteristic group bands (two very strong absorption bands at 6.57 μ and 7.42 μ characteristic of the valence vibrations of —C—NO_2). The hydrazide is soluble in hot water and hot dioxane, and only slightly soluble in methanol, ethanol, or propanol. It is insoluble in chloroform, benzene, and ethyl acetate.

Anal.—Calcd. for $C_{11}H_{15}N_5O_4$: C, 46.97; H, 5.38; N, 24.90. Found: C, 46.91, H, 5.52; N, 25.01.

2-(3′-Nitrophenyl)-trimethylenediamine (XII)—A mixture of 28.1 g. (0.1 mole) of the hydrazide (XI) in 44 ml. of concentrated HCl, 87 g. cracked ice, and 100 ml. of chloroform was diazotized at −5° with 18 g. of sodium nitrite dissolved in 33 ml. of water. After completion of the reaction (*ca.* 30 min.) the chloroform layer was separated and the aqueous layer was extracted twice with 30-ml. portions of chilled chloroform. The combined chloroform extracts were dried over anhydrous calcium chloride. The dried solution of 2-(3′-nitrophenyl)-glutaryl azide was added to 100 ml. of dry toluene and warmed on a steam bath. As soon as the chloroform began to distil out, evolution of nitrogen commenced and continued at a vigorous rate. After all the chloroform had been distilled out, the solution was refluxed strongly for about 1 hr. to complete the decomposition of the azide. To the stirred, hot, dark brown solution of the isocyanate, 60 ml. of concentrated HCl was added cautiously and immediately carbon dioxide began to evolve copiously. After the carbon dioxide evolution had ceased, the reaction mixture was refluxed for 24 hr. and then was concentrated to dryness *in vacuo* on a water bath. The residue of brown crystalline 2-(3′-nitrophenyl)-trimethylenediamine dihydrochloride was suspended in 100 ml. of absolute ethanol, and the suspension was gently refluxed for a few minutes. After cooling to room temperature the salt was filtered and washed with absolute ethanol and absolute ether. After air drying and drying at 80° *in vacuo* for a few hours the crude product weighed 17.5 g. (65% yield) and melted above 275°. A small portion of the dihydrochloride salt was crystallized from methanol–ethyl acetate mixture. The colorless crystalline

product after drying at 80° *in vacuo* melted above 275°.

Anal.—Calcd. for $C_9H_{15}Cl_2N_3O_2$: C, 40.31; H, 5.64; N, 15.67; Cl, 26.45. Found: C, 40.25; H, 5.58; N, 15.85; Cl, 26.15.

The dihydrochloride salt (17.5 g.) was dissolved in 20 ml. of water and the solution was made strongly alkaline with 30% NaOH. The free diamine was extracted with six 15-ml. portions of chloroform, dried, filtered, and distilled *in vacuo* under N_2. The diamine (XII) was thus obtained as a clear yellow oil which on exposure to light turned dark red, b.p. 135–137° (0.05 mm.). Yield: 9.76 g. (77% based on the dihydrochloride salt). Total yield: 50%.

On standing overnight in the refrigerator, the diamine solidified. 2-(3′-Nitrophenyl)-trimethylenediamine (XII) is soluble in warm water and in most organic solvents except for ether, methylcylohexane, and carbon tetrachloride. Because of the pronounced tendency of this diamine to form a carbonate on exposure to the atmosphere its analysis is rather poor but still indicative.

Anal.—Calcd. for $C_9H_{13}N_3O_2$: C, 55.37; H, 6.71; N, 21.53; equiv. wt. 97.61. Found: C, 54.81; H, 6.99; N, 20.83; equiv. wt. 99.25.

The infrared spectra of the freshly prepared diamine possessed characteristic bands in common for the functional groups. $\lambda_{max.}$ ($CHCl_3$): 3.05 μ (medium, N—H stretching vibrations), 3.40 μ (strong, C—H stretching vibrations), 6.29 μ (medium, probably overlapping of N—H deformation absorption and aromatic ring vibrations), 6.56 μ and 7.40 μ (very strong, valence vibrations of —C—NO_2), 12.41 μ and 14.60 μ (medium, out-of-plane deformation vibrations of three adjacent aromatic hydrogens).

2-(3′-Nitrophenyl)-trimethylenedinitrilo Tetraacetic Acid Tetramethyl Ester (XIII)—To a well-stirred suspension of 64.3 g. (0.42 mole) of methyl bromoacetate and 44.5 g. (0.42 mole) of anhydrous Na_2CO_3 in 100 ml. of acetonitrile, a solution of 19.5 g. (0.1 mole) of 2-(3′-nitrophenyl)-trimethylenediamine (XII) in 25 ml. of acetonitrile was added over about 0.5 hr. at 20–25°, while passing nitrogen through the reaction mixture. The reaction mixture was then stirred overnight and later refluxed for another hour. The inorganic salt was filtered and the solvent and excess of methyl bromoacetate were removed under reduced pressure on water bath. The crude product, obtained as a clear brown viscous oil, weighed 46 g. (95% yield) and on TLC (solvent: ethyl acetate) gave one principal spot indicating at least 90% purity. A small sample of the crude amino ester upon distillation in high vacuum, b.p. 210–215° (0.05 mm.), yielded analytically pure substance as a yellow viscous oil. Infrared absorption spectra (CCl_4) $\lambda_{max.}$: 3.32 μ (strong, aliphatic C—H stretching vibrations), 5.74 μ (very strong, C=O stretching vibrations of ester), 6.54 μ and 7.43 μ (strong, —C—NO_2 valence vibrations), 8.38 μ and 9.71 μ (very strong and strong, C—O—C stretching vibrations of acetoxyl group), 14.61 μ (medium, aromatic C—H deformation vibration).

The NMR spectrum of XIII in $CDCl_3$ (60 Mc. TMS reference standard) showed one rather broad peak at δ 3.10 with a shoulder at a higher field, due to the four protons of the two methylene groups, a singlet at 3.48 due to the overlapping of eight protons of four methylene groups, a singlet at 3.69 due to the overlapping of 12 protons of the four OCH_3 groups, and two multiplets each accounting for two aromatic protons centered at 7.53 and 7.95, respectively.

Anal.—Calcd. for $C_{21}H_{29}N_3O_{10}$: C, 52.17; H, 6.05; N, 8.69. Found: C, 52.59; H, 6.24; N, 8.37.

The tetraethyl ester, b.p. 230–234° (0.04 mm.), yellow viscous oil.

Anal.—Calcd. for $C_{25}H_{37}N_3O_{10}$: C, 55.65; H, 6.91; N, 7.79. Found: C, 55.25; H, 6.85; N, 8.09.

2-(3′-Nitrophenyl)-trimethylenedinitrilo Tetraacetic Acid (XIV)—Crude 2-(3′-nitrophenyl)-trimethylenedinitrilo tetraacetic acid tetramethyl ester (XIII) (48.4 g., 0.1 mole) dissolved in 100 ml. of methanol, was directly saponified with a NaOH solution (20 g., 0.5 mole, dissolved in 125 ml. of water). The hydrolysis of the four ester functions was completed within a few minutes, on boiling the reaction mixture. The clear dark red alkaline solution, was treated with active charcoal and refluxed for another 15 min., then cooled, filtered, extracted with ether, and carefully acidified with 42.7 ml. of concentrated HCl (11.7 *M*). The free amino acid (XIV) precipitated from the acidic (pH $\sim$ 1) aqueous solution as tiny particles. The amino acid was filtered, thoroughly washed with water and acetone, and then dried at 80° for a few hours. The amino acid (XIV) obtained in this way as a white, finely divided crystalline powder is already analytically pure and crystallization from a mixture of DMF–water does not change its decomposition point. Yield: 36 g. (84%), m.p. 224–225° dec.

Anal.—Calcd. for $C_{17}H_{21}N_3O_{10}$: C, 47.77; H, 4.95; N, 9.83; equiv. wt. 213.68. Found: C, 47.61; H, 4.85; N, 9.76; equiv. wt. 212.50.

The amino acid is only slightly soluble in hot water and insoluble in most organic solvents except for DMF. 2-(3′-Nitrophenyl)-trimethylenedinitrilo tetraacetic acid was also prepared by a direct adaptation of Dwyer and Garvan's method (12): alkylation of 2-(3′-nitrophenyl)-trimethylenediamine (XII) (0.1 mole), with an excess of sodium chloroacetate (0.6 *M*) in strongly alkaline solution at room temperature. However, longer reaction time (*ca.* 4 days) was necessary and lower yields (*ca.* 40%) were obtained compared with the previous method.

Attempted Preparation of 2-(3′-Amino-2′,4′,6′-triiodophenyl)-trimethylenedinitrilo Tetraacetic Acid—A solution of 4.27 g. (0.01 mole) of the nitrophenyl derivative (XIV) in 35 ml. of 1 *N* HCl, was hydrogenated at room temperature in a Parr low-pressure hydrogenator, using 0.5 g. of 5% Pd/C. After the theoretical quantity of hydrogen for reduction of the nitro group had been absorbed, the solution was filtered to remove the catalyst. The filtrate was used for the iodination step without isolating the intermediate aminophenyl derivative. Employing either iodine monochloride or potassium iododichloride as iodinating agents did not yield the desired aminotriiodophenyl derivative. Titration of an aliquot sample of the iodination reaction mixture, indicated that only one-third of the theoretical quantity of iodine monochloride had been consumed, even after prolonged reaction time (1 week).

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Keyphrases

X-Ray contrast agents—synthesis, X-ray intensity
3-Phenyl-glutaric acid, iodinated derivatives—synthesis
N-Benzyl-iminodiacetic acid, iodinated derivatives—synthesis
NMR spectroscopy—structure
IR spectrophotometry—structure
Toxicity testing—X-ray contrast agents

Assay of Vitamin E in Multivitamin Products Using Thin-Layer Chromatography

By R. R. CRAWFORD, D. C. NARAMORE, and O. K. ESMERIAN

A method for the assay of vitamin E in multivitamin formulas is presented. The sample is saponified and the alpha tocopherol separated from interfering substances by thin-layer chromatography prior to measurement by ferric chloride–α,α'-dipyridyl colorimetry. Assay results of 18 multivitamin formulas comprising three types of dosage forms are in reasonable agreement with label claims. The nature of some interfering substances is discussed.

PRESENT OFFICIAL methods for assaying vitamin E (alpha tocopherol) employ oxidation by either ferric chloride or by ceric sulfate. The amount of alpha tocopherol is determined by the reaction of ferrous ions with α,α'-dipyridyl (red complex) or by titration with ceric sulfate until an excess of ceric ions oxidizes diphenylamine indicator. These methods were critically examined by Lehman before suggesting oxidation by ferric chloride as the method of choice for assaying vitamin E in pharmaceutical products (1). Both of these methods, however, are subject to interference from other reducing materials, among which are antioxidants and vitamin A, included in most formulations. The results from these methods also include responses from the less biologically active nonalpha isomers of tocopherol present in those multivitamin products that use *d*-alpha tocopheryl acetate concentrate or mixed tocopherols concentrate as the vitamin E source.

Early work in this field was directed toward the assay of vitamin E in the presence of only vitamin A, culminating in the hydrogenation procedure reported by Fisher *et al.* (2), and included in the United States Pharmacopeia XVII (3). Paper chromatography has also been applied to vitamin A and E mixtures (4). Recent gas-liquid techniques for identification and estimation of tocopherols have been described (5–7) and applied by Pillsbury *et al.* (8) and by Bowman and West (9) to pharmaceuticals. Several recent references are found pertaining to thin-layer chromatography of tocopherols *per se* (10), in biologic materials (11), in oils and plant tissues (12, 13), and in serum (14). Castrén has reported the determination of vitamin E in pharmaceuticals using TLC separations (silica gel) followed by quantitative estimation of the developed spots by planimetry and visual observation (15, 16).[1]

METHOD

The method developed for multivitamin formulations containing vitamin E in potencies of from 0.15

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[1] For a comprehensive review of vitamin E methodology, see Bunnell, R. H., "The Vitamins," Vol. 6, Academic Press, New York, N. Y., 1967, pp. 261–316.