

pyridine was added slowly 25.5 g. (0.25 mole) of acetic anhydride. The solution was stirred at 0° for 5 hr. and diluted with 1.0 l. of cold water. The organic layer was separated, and the aqueous layer was extracted with diethyl ether. The organic layer and ether extracts were combined and dried over anhydrous Na₂SO₄, and the ether was removed *in vacuo*. The resulting yellow oil was distilled *in vacuo*, and the collected fraction distilled at 113–116°/0.2 mm. The 2-acetoxy-5-methoxybenzaldehyde was collected as a white solid (Table VI).

2-Acetoxy-5-substituted Cinnamaldehydes—The procedure described for 5-substituted-2-methoxycinnamaldehydes was followed.

5-Substituted-2-hydroxycinnamaldehydes—To a cooled (10°) solution of 2-acetoxy-5-methoxycinnamaldehyde (6.6 g., 0.03 mole) in 60 ml. of CHCl₃ was added a solution of Na (0.69 g., 0.03 mole) in 25 ml. of CH₃OH. After complete addition (30 min.), the solution was stirred at 10° for 15 min. and then at room temperature for 1 hr. The reaction mixture was diluted with 100 ml. of water, and the CHCl₃ layer was separated. The aqueous layer was rendered acidic by the addition of dilute H₂SO₄. The solid that formed was collected and washed thoroughly with water. Recrystallization from benzene gave the product as a bright-yellow solid (Table VI).

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Triazenes of Phenylbutyric, Hydrocinnamic, Phenoxyacetic, and Benzoylglutamic Acid Derivatives

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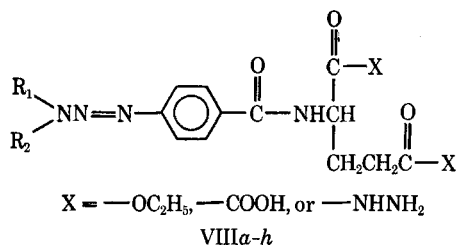
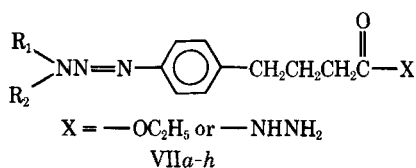
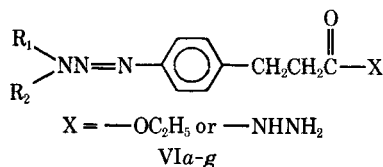
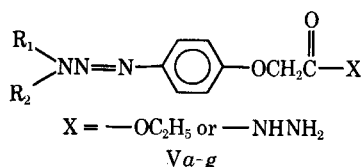
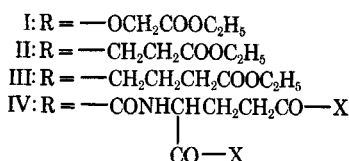
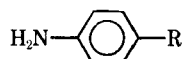
Abstract □ *p*-Dialkyltriazeno derivatives of the ethyl esters and acid hydrazides of phenylbutyric, hydrocinnamic, phenoxyacetic, and benzoylglutamic acids were synthesized. The triazeno phenylbutyric acid derivatives are structurally related to chlorambucil, and the hydrocinnamic and phenoxyacetic acid derivatives are related to other antineoplastic aromatic nitrogen mustards. All of the triazeno groups contained unsubstituted alkyl groups (except for a hydroxyethyl group in a derivative of benzoylglutamic acid). In initial tests of these compounds *versus* mouse lymphatic leukemia L-1210, ethyl *p*-(3-butyl-3-methyl-1-triazeno)hydrocinnamate (VIb) was the most effective compound in increasing the survival time of treated animals. Certain other hydrocinnamic and phenylbutyric acid derivatives caused small increases in survival time.

Keyphrases □ Triazenes of phenylbutyric, hydrocinnamic, phenoxyacetic, and benzoylglutamic acid esters and hydrazides—synthesis, antileukemic activity □ Antileukemic activity—*p*-dialkyltriazene derivatives

After antineoplastic activity was found among triazenoimidazoles (*e.g.*, 1, 2), it seemed reasonable to suppose that combining substituted triazeno groups with

structural moieties that can presumably serve as carrier groups might also produce derivatives having antineoplastic activity. Studies of aromatic nitrogen mustards included a series of phenylalkanoic acid derivatives. Of the initial series, chlorambucil, 4-*p*-[bis(2-chloroethyl)amino]phenylbutyric acid, was the most effective derivative in inhibiting the transplanted Walker rat carcinoma (3, 4) and proved to be a clinically useful agent (*e.g.*, 5, 6). The enhanced activity of chlorambucil was attributed (3, 4) to the constitution of its phenylbutyric acid moiety rather than to its chemical reactivity or physical properties, and the phenylbutyric acid portion was used as a carrier for other cytotoxic groups (7).

Nitrogen mustard derivatives of hydrocinnamic acid (3) and of phenoxyalkanoic acids (8, 9)—notably the phenoxypropionic acid derivative—were also active antineoplastic agents, and activity was retained in certain ester derivatives (3, 9). The ethyl esters of phenylbutyric acid, hydrocinnamic acid, and phenoxyacetic acid were chosen, therefore, for attachment of triazeno



groups at the *para*-position. Diethyl *p*-aminobenzoylglutamate, the ester of a folic acid moiety, was also chosen for conversion to triazeno derivatives¹.

Representative triazeno derivatives (V–VIII, Table I) of the four aromatic moieties were prepared by diazotizing an aromatic amine (I–IV) and then adding an aliphatic amine in sufficient quantity to raise the pH to 9–10 or above. Certain liquid triazenes were sufficiently volatile and stable to be distilled; the other triazenes were purified by other conventional methods. Compound VIII_f, the only triazeno derivative of an acid, precipitated from a slightly acidic solution as the mono-(dibutylammonium) salt. The acid hydrazides (V_f–g, VI_f–g, VII_g–h, and VIII_g–h) were obtained by reactions of hydrazine with the dimethyltriazeno or butylmethyltriazeno derivatives of the ethyl esters.

All of the triazenes except V_c and VIII_h were tested on a daily treatment schedule against lymphatic leukemia L-1210 in mice in accordance with previously described procedures (13, *cf.*, footnotes *a–d* of Table I in Reference 12). The highest increases in lifespan resulted from treatment with ethyl *p*-(3-butyl-3-methyl-1-triazeno)hydrocinnamate (VI_b, NSC-77587). In several separate experiments, increases in lifespan of 40–80% were re-

corded at doses of 250–600 mg./kg./day (Table II). The highest dose was acutely toxic in one of two tests, and the average difference in weight change between treated and untreated animals was about 3–4 g. in most tests at these dose levels.

Initial tests of the phenylbutyric acid derivatives and of the remaining hydrocinnamic acid derivatives indicated that some of these compounds can also cause small increases in lifespan. Thus, modest increases in survival time were observed in two or more tests of the dimethyltriazeno derivatives of the acid hydrazides (VI_f and VII_g) and after administration of ethyl 4-[*p*-(3,3-dimethyl-1-triazeno)phenyl]butyrate (VII_a) at three dosages. Data from tests of these and of other compounds that produced an increase of 25% or more in lifespan in at least one test are also summarized in Table II. Although Compound VI_c caused a significant increase in lifespan in a single test at 375 mg./kg./day, this apparent activity was not confirmed by a second test at a slightly higher dose (400 mg./kg./day).

Extensive testing of the derivatives not included in Table II might reveal activity, but none of these compounds caused significant increases in lifespan in the preliminary standard L-1210 tests. When only one dose of a compound is administered and the ratio of survival times is near 100%, a possible explanation is that the potential increase in survival time of treated animals due to cell kill is counterbalanced by chronic toxicity to the host. A lower dose might then produce a higher *T/C* ratio (*e.g.*, VI_f, Table II). Except for those compounds specifically mentioned here, the remaining hydrocinnamic (VI) and phenylbutyric (VII) acid derivatives, the phenoxyacetic acid derivatives (V), and the benzoylglutamic acid derivatives (VIII) were tested at two or more daily dosages; the highest dose was either a toxic dose or the maximum administered dose (400 or 500 mg./kg./day). The exceptions were: Compounds VI_d and VIII_e were tested at only one dose (400 mg./kg./day), but the slight difference in weight change between treated and control animals indicated that their failure to increase lifespan was not due to toxicity; the highest doses of Compounds V_g and VI_g administered were just below toxic doses in tests *versus* adenocarcinoma 755 (Ca 755); the only dose (30 mg./kg./day) of VII_h was one-half the Ca 755 toxic dose; and of the two doses (200 and 100 mg./kg./day) of VIII_g administered, the highest dose was the same as that which caused a large difference in weight change in a Ca 755 test.

EXPERIMENTAL²

Aromatic Amine Precursors (I–IV)—Ethyl *p*-nitrophenoxyacetate [m.p. 74–75°; lit. 75–76° (14), 74–75° (15)] was prepared by esterifying the nitro acid in ethanolic hydrogen chloride. Catalytic hydrogenation (5% palladium-on-charcoal in ethanol) of the nitro ester and recrystallization of the product from a benzene-petroleum ether mixture gave ethyl *p*-aminophenoxyacetate (I) (16) in yields of 85–97%; m.p. 56–57°.

Ethyl *p*-aminohydrocinnamate (II) (17, 18) was prepared by two methods. First, *p*-aminohydrocinnamic acid (m.p. 133–134°), obtained in yields of 80–95% by reduction of *p*-nitrocinnamic acid by

¹ This type of structure was previously utilized for the attachment of the nitrogen mustard group (10, 11).

² Unless otherwise stated, melting temperatures were determined on a Kofler Heizbank apparatus (gradiently heated bar). Those designated "cap." were determined in a capillary tube.

Table I—Triazenes (V-VIII)

Number	Compound		X	Method of Purification ^e	Yield, %	Melting Point or Boiling Point ^b	n_D^{20} (°C) or $[\alpha]_D^{25}$	Empirical Formula	Analysis, %	
	R ₁	R ₂							Calc.	Found
Phenoxyacetic Acid Derivatives										
Va	CH ₃	CH ₃	OC ₂ H ₅	C	86	126–127 ^{o,d} (0.15 mm.)	1.5748 (25°)	C ₁₂ H ₁₇ N ₃ O ₂	C, 57.36 H, 6.82 N, 16.72	C, 57.54 H, 6.96 N, 16.83
Vb	CH ₃	<i>n</i> -C ₄ H ₉	OC ₂ H ₅	A	88		1.5543 (20°)	C ₁₅ H ₂₃ N ₃ O ₂	C, 61.41 H, 7.91 N, 14.32	C, 61.37 H, 7.83 N, 14.34
Vc	CH ₃	iso-C ₄ H ₉	OC ₂ H ₅	F	72		1.5517 (26°)	C ₁₅ H ₂₃ N ₃ O ₂	C, 61.41 H, 7.91 N, 14.32	C, 61.48 H, 7.89 N, 14.63
Vd	CH ₃	C ₆ H ₁₁	OC ₂ H ₅	F	40	40–41°	1.5679 (26°) ^e	C ₁₇ H ₂₅ N ₃ O ₂	C, 63.92 H, 7.89 N, 13.15	C, 63.97 H, 7.90 N, 13.34
Ve	R ₁ R ₂ N = pyrrolidinyl		OC ₂ H ₅	D ^f (EtOH–H ₂ O)	89	50–51°		C ₁₄ H ₁₉ N ₃ O ₂	C, 60.64 H, 6.91 N, 15.15	C, 60.87 H, 6.88 N, 15.16
Vf	CH ₃	CH ₃	—NHNH ₂	D(EtOH)	92	145–146°		C ₁₀ H ₁₃ N ₃ O ₂	C, 50.65 H, 6.37 N, 29.52	C, 50.51 H, 6.34 N, 29.46
Vg	CH ₃	<i>n</i> -C ₄ H ₉	—NHNH ₂	D(C ₆ H ₆ –hexane)	90	78–80°		C ₁₃ H ₂₁ N ₃ O ₂	C, 55.89 H, 7.58 N, 25.07	C, 56.02 H, 7.47 N, 25.16
Hydrocinamic Acid Derivatives										
VIa	CH ₃	CH ₃	OC ₂ H ₅	C	85	128–130° (0.05 mm.)	1.5645 (25°)	C ₁₃ H ₁₉ N ₃ O ₂	C, 62.62 H, 7.68 N, 16.90	C, 62.62 H, 7.74 N, 16.90
VIb	CH ₃	<i>n</i> -C ₄ H ₉	OC ₂ H ₅	E	74		1.5468 (23.5°)	C ₁₆ H ₂₃ N ₃ O ₂	C, 65.94 H, 8.65 N, 14.21	C, 65.65 H, 8.63 N, 14.21
VIc	CH ₃	<i>sec</i> -C ₄ H ₉	OC ₂ H ₅	E	79		1.5401 (26°)	C ₁₆ H ₂₃ N ₃ O ₂	C, 65.94 H, 8.65 N, 14.42	C, 65.49 H, 8.87 N, 14.47
VI d	CH ₃	C ₆ H ₁₁	OC ₂ H ₅	A	85		1.5627 (24°)	C ₁₈ H ₂₇ N ₃ O ₂	C, 68.11 H, 8.58 N, 13.25	C, 67.95 H, 8.58 N, 13.23
VIe	R ₁ R ₂ N = pyrrolidinyl		OC ₂ H ₅	A	96	39–40°		C ₁₅ H ₂₁ N ₃ O ₂	C, 65.43 H, 7.69 N, 15.26	C, 65.62 H, 7.80 N, 15.23
VI	CH ₃	CH ₃	—NHNH ₂	D(EtOH–hexane)	83	94–95°		C ₁₁ H ₁₇ N ₃ O	C, 56.14 H, 7.28 N, 29.76	C, 56.08 H, 7.09 N, 29.72
VIg	CH ₃	<i>n</i> -C ₄ H ₉	—NHNH ₂	D(C ₆ H ₆ –hexane)	87	51–52°		C ₁₄ H ₂₃ N ₃ O	C, 60.62 H, 8.36 N, 25.25	C, 60.52 H, 8.32 N, 25.10
Phenylbutyric Acid Derivatives										
VIIa	CH ₃	CH ₃	OC ₂ H ₅	A	97		1.5603 (21°)	C ₁₄ H ₂₁ N ₃ O ₂	C, 63.85 H, 8.04 N, 15.96	C, 64.07 H, 8.08 N, 16.10
VIIb	CH ₃	<i>n</i> -C ₄ H ₉	OC ₂ H ₅	B	84		1.5427 (24°)	C ₁₇ H ₂₇ N ₃ O ₂	C, 66.85 H, 8.91 N, 13.76	C, 66.62 H, 8.73 N, 13.93
VIIc	CH ₃	iso-C ₄ H ₉	OC ₂ H ₅	E	71		1.5418 (24°)	C ₁₇ H ₂₇ N ₃ O ₂	C, 66.85 H, 8.91 N, 13.76	C, 67.06 H, 8.50 N, 13.90

Table I—(Continued)

Number	Compound		X	Method of Purification ^a	Yield, %	Melting Point or Boiling Point ^b	<i>n</i> _D (°C) or <i>l</i> _D ²⁰	Empirical Formula	Analysis, %	
	R ₁	R ₂							Calc.	Found
VIII d	CH ₃	<i>sec</i> -C ₄ H ₉	OC ₂ H ₅	E	76		1.5416 (26°)	C ₁₇ H ₂₇ N ₃ O ₂	66.85 8.91 13.76	66.67 8.84 14.14
VIII e	CH ₃	C ₆ H ₁₁	OC ₂ H ₅	E	82 ^c		1.5555 (24°)	C ₁₉ H ₂₉ N ₃ O ₂	68.85 8.81 12.68	69.08 8.92 12.60
VIII f	<i>n</i> -C ₄ H ₉	<i>n</i> -C ₄ H ₉	OC ₂ H ₅	B	93		1.5319 (25°)	C ₂₀ H ₃₃ N ₃ O ₂	69.12 9.58 12.10	69.25 9.36 12.20
VIII g	CH ₃	CH ₃	—NHNH ₂	D(EtOH-P.E.)	68	118–120°		C ₁₂ H ₁₉ N ₅ O	57.81 7.68 28.09	58.08 7.71 27.90
VIII h	CH ₃	<i>n</i> -C ₄ H ₉	—NHNH ₂	D(C ₆ H ₆ -hexane)	92	56–58°		C ₁₈ H ₂₅ N ₆ O	61.83 8.65 24.04	62.02 8.55 23.91
Benzoylglutamic Acid Derivatives ^b										
VIII a	CH ₃	CH ₃	OEt	D(EtOH-H ₂ O)	73	77°	-12.7	C ₁₈ H ₂₅ N ₄ O ₅	57.12 6.93 14.80	56.98 6.79 15.00
VIII b	CH ₃	CH ₃	OEt	D(EtOH-H ₂ O)	78	114–115°	DL	C ₁₈ H ₂₆ N ₄ O ₅	57.12 6.93 14.80	57.16 7.01 14.83
VIII c	CH ₃	<i>n</i> -C ₄ H ₉	OEt	D(C ₆ H ₆ -hexane)	80	46–48°	-10.0	C ₂₁ H ₃₂ N ₄ O ₅	59.98 7.67 13.32	59.80 7.49 13.27
VIII d	<i>n</i> -C ₄ H	<i>n</i> -C ₄ H ₉	OEt	E	44	44–46°	-9.3	C ₂₄ H ₃₈ N ₄ O ₅	62.31 8.28 12.11	62.59 8.08 11.95
VIII e	CH ₃	—CH ₂ CH ₂ OH	OEt	E	73		-10.6	C ₁₉ H ₂₈ N ₄ O ₆	55.88 6.91 13.72	55.59 6.91 13.54
VIII f	<i>n</i> -C ₄ H ₉	<i>n</i> -C ₄ H ₉ mono(dibutylammonium) salt	OH		71	136–142°	+17.2	C ₂₀ H ₃₀ N ₄ O ₅ C ₈ H ₁₉ N	62.78 9.21 13.08	63.12 9.22 12.91
VIII g	CH ₃	CH ₃	—NHNH ₂	D(DMF-H ₂ O)	64	208–210°	+32	C ₁₄ H ₂₂ N ₆ O ₃	47.98 6.33 32.01	48.84 6.37 31.96
VIII h	CH ₃	<i>n</i> -C ₄ H ₉	—NHNH ₂	D(EtOH)	53	192°	+29	C ₁₇ H ₂₈ N ₆ O ₃	52.03 7.21 28.55	51.99 7.17 28.44

^a Methods of purification are defined in the general experimental procedure for the triazenes of phenoxyacetic acid ester, etc.; further details for the benzoylglutamate triazenes are given in the Experimental part. P.E. = petroleum ether (b.p. 30–60°); DMF = dimethylformamide. ^b Boiling point when pressure in the distillation apparatus is stated. Melting points of Vd, VIe, VIIIh, VIIIc, and VIIIe were determined in capillary tubes; cf. Footnote 2. ^c *l*_D²⁰ for benzoylglutamic acid derivatives. EtOH was the solvent for VIIIa–f and dimethylformamide for VIIIg–h; c = 1.5–1.6. ^d Obtained as a liquid; solidified during storage at low temperatures (m.p. 29–31°). ^e Obtained as a liquid; solidified during storage at low temperatures. ^f Product precipitated from reaction mixture; ethyl acetate extraction omitted. ^g Yield after Method A only; IR spectrum and analyses satisfactory. ^h Derivatives of L-glutamic acid except for VIIIh.

Table II—Results of Tests of Some Triazenes against L-1210^{a, b}

Compound	Dose, mg./kg./day	Schedule ^c	Mortality	Difference in Average Weight Change, T/C	Survival Time, T/C, %
Ethyl <i>p</i> -(3-butyl-3-methyl-1-triazeno)hydrocinnamate (VIb)	400	1	0/6	-2.1	108
	400	1-9	0/6	-4.0	161
	400	1-9	0/6	-6.0	130
	375	1-d	0/6	-4.4	137
	250	1-d	0/6	-2.5	142
	187	1-d	0/6	-2.4	117
	600	1-9	1/6	-4.3	179
	400	1-9	0/6	-3.4	162
	266	1-9	0/6	-2.1	128
	177	1-9	0/6	-1.3	117
	600	1-7	5/6		
	400	1-7	0/6	-3.3	171
	266	1-7	0/6	-3.3	160
	177	1-7	0/6	-3.1	144
	<i>p</i> -(3,3-Dimethyl-1-triazeno)-hydrocinnamic acid hydrazide (VI _f)	67	1-d	0/6	-6.4
45		1-d	0/6	-4.2	141
45		1-d	0/6	-5.4	132
4-[<i>p</i> -(3,3-Dimethyl-1-triazeno)-phenyl]butyric acid hydrazide (VIIg)	30	1-d	0/6	-1.7	114
	85	1-d	0/6	-5.9	82 ^t
	45	1-d	0/6	-3.3	128
	45	1-d	0/6	-3.4	142
	42	1-d	0/6	-4.5	129
	42	1-d	0/6	-3.3	130
	63	1-9	0/6	-4.3	146
	42	1-9	0/6	-2.9	127
	28	1-9	0/6	-2.7	111
Ethyl 4-[<i>p</i> -(3,3-dimethyl-1-triazeno)phenyl]butyrate (VIIa)	18	1-9	0/6	-1.6	103
	250	1-d	5/6		^t
	125	1-d	0/6	-2.8	92
	100	1-9	1/5	-3.3	130
	75	1-9	0/6	-2.7	134
	50	1-9	0/6	-1.7	133
Ethyl <i>p</i> -(3,3-dimethyl-1-triazeno)-hydrocinnamate (VIa)	400	1-9	0/6	-2.9	90
	250	1-d	0/6	-1.8	105
	125	1-9	0/6	-1.6	115
Ethyl 4-[<i>p</i> -(3-butyl-3-methyl-1-triazeno)phenyl]butyrate (VIIb)	83	1-9	0/6	-1.8	125
	500	1-d	0/6	-4.1	97
	250	1-d	0/6	-3.9	121
	187	1-9	0/6	-1.4	128 ^d
	125	1-d	0/6	-4.2	134
	125	1-9	0/6	-1.7	119
Ethyl 4-[<i>p</i> -(3- <i>sec</i> -butyl-3-methyl-1-triazeno)phenyl]-butyrate (VII _d)	84	1-9	0/6	-2.0	115
	400	1-d	1/6	-2.8	112
Ethyl <i>p</i> -(3- <i>sec</i> -butyl-3-methyl-1-triazeno)hydrocinnamate (VIc)	200	1-8	0/6	-2.0	130
	375	1-d	0/6	-2.6	147
Diethyl <i>N</i> -[<i>p</i> -(3-butyl-3-methyl-1-triazeno)benzoyl]glutamate (VIIIc)	400	1-d	0/6	-1.0	119
	250	1-d	0/6	-1.6	108
	500	1-d	0/6	-0.6	126
	375	1-d	0/6	-0.4	108
	250	1-d	0/6	-1.2	121

^a Derivatives of V-VIII that increased lifespan by 25% or more in at least one test; ^t = toxic. Except as noted, fresh solutions or suspensions of the triazenes were prepared each day before injection. ^b Further explanation of testing *versus* L-1210 is given in References 12 (footnotes a-d of Table I) and 13. ^c 1 means administration of a single dose on Day 1 (24 hr. after implantation of 10⁵ L-1210 cells); 1-9, 1-8, 1-7, and 1-d mean daily administration on Days 1-9, 1-8, 1-7, and from Day 1 until Day 15 or until death, respectively. ^d Administered as a stable compound at this dose; solutions prepared on Days 0 and 6 (13).

the method of Skinner *et al.* (19), was esterified with refluxing (2 hr.) ethanolic hydrogen chloride. The solvent was evaporated, the residue was dissolved in dilute aqueous sodium hydroxide (pH 9), and the ester was extracted with ethyl acetate. Removal of the solvent and distillation of the residue gave the amino ester (II) as a colorless oil which solidified during storage at about 5°; yield, 83%; b.p. 99-100° (0.09 mm.); n_D^{25} 1.5360. The second method consisted of catalytic hydrogenation (5% palladium-on-charcoal) of ethyl *p*-nitrocinnamate in ethanol and distillation after removal of the solvent; yield of II, 80-90%.

Ethyl *p*-aminophenylbutyrate (II) (20) was also prepared by two routes. First, *p*-nitrophenylbutyric acid was obtained by the nitration procedure of Reppe (21) in 22% yield after two recrystallizations: m.p. 92-93° [lit. 95° (21), 92° (3), 92-93° (22)]. (Some of the *ortho*-isomer was also isolated.) The nitro acid was esterified with refluxing (1.5 hr.) ethanolic hydrogen chloride, the solvent was removed *in vacuo*, and the residual oil was distilled: yield, 76%; b.p. 121-123° (0.09 mm.); n_D^{25} 1.5238. The boiling point, refractive index, and IR spectrum were unchanged after redistillation; only the *para*-isomer

was observable in the NMR spectrum, and assay by VPC showed the redistilled product to be 99.5% *para*-isomer.

Anal.—Calc. for C₁₂H₁₅NO₄: C, 60.75; H, 6.37; N, 5.91. Found: C, 60.84; H, 6.21; N, 5.96.

Catalytic hydrogenation (palladium-on-charcoal in ethanol) of the nitro ester and distillation of the crude product gave a yield of 92% of III: b.p. 104-106° (0.1 mm.); n_D^{25} 1.5297.

Anal.—Calc. for C₁₂H₁₇NO₂: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.81; H, 8.24; N, 6.92.

The second route to III consisted of the synthesis of *p*-aminophenylbutyric acid (m.p. 130-132°) from succinic anhydride and acetanilide (23, 24) and esterification of the amino acid in the usual manner. The IR spectrum and refractive index were identical with those of III obtained by the first route.

DL-p-Aminobenzoylglutamic acid [m.p. 197-198° (cap.); lit. (25) 198-199°] was prepared by hydrogenation (5% palladium-on-charcoal in ethanol) of the nitro derivative (25). Esterification of the acid with refluxing (3.5 hr.) ethanolic hydrogen chloride gave diethyl

DL-*p*-aminobenzoylglutamate³ (DL-IV, X = OC₂H₅): m.p. 117–119° (cap.).

Anal.—Calc. for C₁₆H₂₂N₂O₅: N, 8.66. Found: N, 8.76.

Diethyl L-*p*-aminobenzoylglutamate (L-IV, X = OC₂H₅) (26, 27) was prepared similarly from L-*p*-aminobenzoylglutamic acid (L-IV, X = OH).

Triazenes of Esters of Phenoxyacetic, Hydrocinnamic, and Phenylbutyric Acids (Va–e, VIa–e, and VIIa–f)—The triazenes were routinely protected from light during the preparation and purification procedures and were prepared under an atmosphere of nitrogen. The aromatic amine (I–III) in 1 *N* hydrochloric acid (3–4 ml./mmole) was diazotized at 0–5° by adding, dropwise during 5–30 min., a solution of sodium nitrite (1.1 mmoles/mmole of aromatic amine) in a volume of water convenient for the size of the preparation (usually 10–20 ml./g.). The solution was stirred at 0–5° for 0.5–1 hr. before the excess nitrite was decomposed with sulfamic acid. The amine to be coupled was then added quickly in sufficient quantity to raise the pH of the solution to 9–10 or above, and stirring at 0–5° was continued for 0.5–2 hr. (typically 1 hr.). The triazene was extracted from the aqueous mixture with ethyl acetate. The ethyl acetate extract was washed well with aqueous sodium chloride solution or successively with 1 *N* hydrochloric acid (to facilitate removal of extracted amine), aqueous sodium bicarbonate, and aqueous sodium chloride. The ethyl acetate solution was dried (MgSO₄), treated with activated carbon, and freed of solvent *in vacuo*. The residual triazene was dried (P₂O₅) *in vacuo* at room temperature.

The products of some preparations were pure, as adjudged by analytical data and TLC, without further treatment; other compounds were obtained analytically pure after redissolution of the products in ethyl acetate or ether and repetition of the washing and drying operations. The purification procedures (Table I) to which the initially isolated triazenes were subjected were the following: (A) no further treatment; (B) redissolution in ethyl acetate or ether and repetition of the washing and drying operations; (C) distillation under reduced pressure; (D) recrystallization; (E) chromatography on magnesia–silica gel⁴ and elution with petroleum ether, hexane, or cyclohexane and then with one of these solvents containing small percentages of acetone; (F) stirring of the dry ethyl acetate extract with a mixture of magnesia–silica gel and activated carbon followed by treatment of the ethyl acetate residue in hexane solution with a portion of magnesia–silica gel (Vd) or in methanol with activated carbon (Vc).

The preparation of ethyl 4-[*p*-(3-*sec*-butyl-3-methyl-1-triazeno)-phenyl]butyrate (VIIId) illustrates both the general procedure and purification by chromatography. A solution of 2.8 g. of sodium nitrite in 50 ml. of water was added during 0.5 hr. to a cold (0–5°) solution of 7.5 g. of ethyl 4-(*p*-aminophenyl)butyrate in 150 ml. of 1 *N* hydrochloric acid; then the following operations were performed successively: the mixture was stirred for 0.5 hr., sufficient sulfamic acid was added to destroy excess nitrite, 45 ml. of *N*-methyl-*sec*-butylamine was added, and the resulting solution (still at 0–5°) was stirred for 1 hr. and then extracted with two 250-ml. portions of ethyl acetate. The ethyl acetate solution (combined portions) was washed successively with two 100-ml. portions of 1 *N* hydrochloric acid, saturated aqueous sodium bicarbonate, and aqueous sodium chloride and was then dried (MgSO₄) and treated with activated carbon. Evaporation of the solvent *in vacuo* left 10.6 g. (96%) of an orange oil; TLC (80 mcg. of sample on silica gel, 95:5 benzene–ethyl acetate, detection by UV and UV-Ultraphor⁵), prominent spot of the triazene plus a small amount of a contaminant at the origin. A hexane solution of the crude product was chromatographed on a column prepared from 100 g. of magnesia–silica gel and hexane. Elution with 500 ml. of hexane gave 8.4 g. (76%) of faintly yellow oil; *n*_D²⁰ 1.5416. Further elution with 500 ml. of 99.5:0.5 hexane–acetone gave 0.89 g. (8%) of orange oil that was identical, according to TLC and IR, with the hexane-eluted product.

***N*-[*p*-(3,3-Dialkyl-1-triazeno)benzoyl]glutamates (VIIIa–f)**—The procedure was similar to that described for the triazenes of ethyl

hydrocinnamate and related esters, except that: (a) the solution of diethyl *p*-aminobenzoylglutamate (IV, X = OC₂H₅) or the parent acid (IV, X = OH) in 1 *N* hydrochloric acid was added (during approximately 1 hr.) to the aqueous solution of sodium nitrite, and (b) the amount of water used in the sodium nitrite solution was larger (typically, 35 ml./g.). Except for Compound VIII_f, the triazenes precipitated from the reaction mixtures. Compound VIII_a precipitated as a solid, and Compound VIII_b was induced to solidify by adding ethanol to the reaction mixture. These two compounds were isolated by filtration and washed with water. The others precipitated as gums or oils and were extracted from the reaction mixtures with ether (VIII_c and VIII_d) or ethyl acetate (VIII_e). The reaction mixture containing VIII_f was washed with ether, and VIII_f was precipitated by lowering the pH. Precipitation began at pH 6.0–5.8, and the pH was not allowed to fall below 5.2. Compounds VIII_a–*c* were recrystallized (Table I).

The ether extract (preceding paragraph) containing VIII_d was freed of solvent, the residual oil was stirred with two portions of cold hexane, the hexane layers were decanted, the oil layer was stirred with a third portion of hexane, and this mixture was then stored overnight at 5°. The solidified triazene was removed by filtration, washed with hexane, and treated in ether solution with activated carbon. The viscous oil remaining after evaporation of the ether was leached with hot petroleum ether. Evaporation of solvent from the petroleum ether extract left a light-yellow oil that solidified: m.p. 44–46°.

The ethyl acetate extract containing VIII_e was washed successively with 1 *N* hydrochloric acid, aqueous sodium bicarbonate, and aqueous sodium chloride and was dried (MgSO₄). The light-brown oil remaining after evaporation of the solvent was reprecipitated from benzene solution with hexane and treated in methanol solution with activated carbon; the residual oil (5.6 g.) obtained by evaporation of the methanol was chromatographed on magnesia–silica gel (60 g.). Elution with 400-ml. portions of hexane containing 0.5, 1, 2, 4, 8, and 16% acetone gave negligible amounts of product; elution with two 400-ml. portions of 32% acetone–hexane then gave 4.70 and 0.67 g., respectively, of a pale-yellow oil (VIII_e, 73% yield); TLC (silica gel, 1:3 benzene–ethyl acetate), 1 spot; UV_{max}. (ε × 10⁻³) in phosphate buffer (pH 7) 223 (9.6), 322 (18.1).

Hydrazide Derivatives (Vf–g, VIg–h, VIIg–h, and VIIIg–h)—The ester derivative of a triazene was cooled in an ice bath, hydrazine was added, and ethanol was then added in sufficient quantity to form a homogeneous reaction mixture. The solution was stirred at room temperature, and the disappearance of the ester was monitored by TLC. During the preparation of the higher melting acid hydrazides, the reaction mixture became a solid mass. The usual reaction times were 24 or 48 hr. The excess hydrazine and the ethanol were then evaporated *in vacuo* (regardless of whether the reaction mixture was solid or liquid), and the residue was recrystallized (Table I). The triazene derivatives were protected from light during the preparation and purification operations.

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³ This compound is reported in the English translation of a paper by Alekseeva and Pushkareva (10); however, the melting points tabulated for the diethyl ester and its two precursors are in agreement with those of the L-isomers.

⁴ Florisil, The Floridin Co.

⁵ Ultraphor WT, an optical whitening agent, BASF Colors and Chemicals, Inc., Charlotte, N. C.

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Rheological and Sensory Evaluation of Work Softening and Recovery of Pharmaceutical White Soft Paraffins

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Abstract □ Continuous shear and creep viscometry were used to investigate the effect of work softening and recovery on the rheological properties of four grades of white soft paraffin BP. In continuous shear, using a cone and plate viscometer, the apparent viscosities of two samples increased with recovery time after working, which indicated partial structural recovery. Apparent viscosities of unworked samples were generally lower than those of the worked samples due to elastic recovery and/or sample fracture of the unworked samples during shear in the viscometer. Viscoelastic results were analyzed to obtain initial elastic compliances and residual viscosities. These data indicated that the loss of consistency during working and recovery after working were mainly viscous phenomena. Continuous spectra of retardation times derived for worked and unworked soft paraffins showed that working caused little irreversible structural breakdown. Structural recovery, indicated by concentrations of retardation mechanisms in the continuous spectra, occurred for at least 240 hr. after work softening. A sensory evaluation of white soft paraffin in different states of working was correlated with discrete viscoelastic parameters and continuous shear yield stresses of the material; no correlation was found with continuous shear apparent viscosity data.

Keyphrases □ White soft paraffin, rheology—work softening and recovery effects □ Rheological properties, white soft paraffin—continuous shear and creep viscometry □ Viscometry, continuous shear, creep—white soft paraffin rheology □ IR spectrophotometry—structure, work softening of soft paraffins

White soft paraffin BP, which is used as an excipient in many pharmaceutical and cosmetic ointments, consists of a three-dimensional crystalline matrix embedded in a colloidal gel of liquid and amorphous hydrocarbons (1). When the material shears, whether during manu-

facture, viscometry, or in application to the skin, the structure is broken down by rupture of bonds within the material and the consistency decreases as the material work softens. Few experiments have been reported concerning the effect of work softening on the rheological properties of white soft paraffin. Haighton (2) and Shama and Sherman (3) investigated work softening of margarine and butter, which are similar in consistency to white soft paraffin. Tsagareishvili *et al.* (4) reported that work softening of soft paraffin caused up to 90% breakdown of bonds within the material, that it reduced the viscosity, and that the cycloparaffin content, measured by IR spectroscopy, increased.

In manufacturing processes involving cold working of materials containing significant amounts of white soft paraffin, the final product will not have its maximum possible structure because of the working procedure. The product consistency will vary, depending on: (a) the initial structural condition of the material, for example, melted and cooled or worked when cold; (b) the degree of work softening; (c) the type or grade of material; and (d) the age of the finished product. The first two variables are, to some extent, under the control of the manufacturer. The effect of grade variation on the rheological properties of unworked white soft paraffin has been investigated by continuous shear and creep viscometry (5). The present study investigated the effect of work softening and recovery on the rheological properties of four grades of white soft paraffin (factors c and d).