C. *O*-**Malonyl-L-serine.**—Finely powdered L-serine (3.15 g, 0.03 mol) was added to a solution of 60 ml of acetone and 3.6 ml of 60% perchloric acid. The resulting solution was added over a 3-min period to a solution of 30 ml (0.27 mol) of malonyl chloride in 55 ml of acetone. During these additions and subsequent incubation for 10 min, the reaction mixture was stirred vigorously and maintained at 30° by use of an ice bath when necessary. The reaction mixture was then cooled to 0° and subsequently maintained at this temperature. Water was added to a total volume of 250 ml and the solution extracted twice, each time with 500 ml of ether. The subsequent procedure was the same as that described for *O*-malonylhomoserine. The product appeared at approximately the same elution volume as did *O*-malaonylhomoserine. The product was crystallized from water to yield 2.29 g (39% yield from serine) of product, which had mp 163–165° after recrystallization from water–ethanol–ether (45:45:10): nmr δ 4.15 (t, 0.80, J = 4 Hz, CH), 4.64 (d, 2.20, J = 4 Hz, CH_2O). *Anal.* Calcd for $C_6H_9NO_6$: C, 37.68; H, 4.75; N, 7.33. Found: C, 37.85; H, 4.84; N, 7.23.

D. *O*-**Succinyl-L-serine.**—To 100 ml of 1,4-*p*-dioxane containing 5 ml of 60% perchloric acid was added 3.15 g (0.03 mol) of finely powdered L-serine. This solution was added over a period of 10 min to 42 ml (0.38 mol) of succinyl chloride. During these additions and subsequent incubation for 1 hr, the reaction mixture was stirred vigorously and maintained at 30° by use of an ice bath when necessary. The reaction mixture was then cooled to 0° and subsequently maintained at this temperature. The relatively slow rate of hydrolysis of succinyl chloride permitted its efficient extraction with ether as follows. Ether (500 ml) was added to the reaction mixture, followed by 200 ml of water. This mixture was immediately shaken vigorously for 3 sec in a separatory funnel, and the aqueous phase was drawn off. Extraction of the ether phase with water was repeated. These extractions were performed rapidly so that most of the succinyl chloride could be extracted before it was hydrolyzed to succinic acid. The aqueous phases were then combined, extracted five times (each time with 500 ml of ether), and titrated to pH 6.5 with 1 *M* KOH. The subsequent procedure was the same as that described for *O*-malonylhomoserine with the following exception. The product was eluted with 0.75 *M* formic acid, appearing between 2.5 and 5.0 l. This fraction was lyophilized to dryness and the resulting powder crystallized from water–ethanol–ether (45:45:10) to yield 1.18 g (19% yield from serine), mp 156.5–157.5°.[12] The compound turned brown near its melting point: nmr δ 2.72 (s, 4.04, CH_2CH_2), 4.14 (t, 0.81, J = 4 Hz, CH), 4.60 (d, 2.15, J = 4 Hz, CH_2O). *Anal.* Calcd from $C_7H_{11}NO_6$: C, 40.96; H, 5.41; N, 6.83. Found: C, 41.10; H, 5.66; N, 6.91.

E. *O*-**Oxalyl-L-serine.**—This compound proved to be the most difficult to synthesize, probably because of its instability (Figure 1). However, a small amount of the compound was synthesized by the same procedure as described for *O*-malonylserine.

Registry No.—*O*-Oxalyl-L-homoserine, 4096-48-4; *O*-malonyl-L-homoserine, 26189-67-3; *O*-malonyl-L-serine, 26189-68-4; *O*-succinyl-L-serine, 21959-23-9.

Acknowledgment.—The authors are indebted to Dr. W. C. Alford, National Institutes of Health, Bethesda, Md., for the elemental analyses, and to Dr. Carl B. Storm, Howard University, Washington, D. C., for assistance in the determination and interpretation of nmr spectra.

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[12] A melting point of 173–174° has been reported by S. Guggenheim and M. Flavin [*J. Biol. Chem.*, **244**, 3722 (1969)], for *O*-succinyl-L-serine prepared by a method involving the reaction of *N*-benzyloxycarboxyl-L-serine with succinic anhydride. Drs. Guggenheim and Flavin kindly supplied a sample of their preparation of *O*-succinyl-L-serine which had been stored at −30° since its preparation in March 1967. In our hands, mp 157–158.5° was determined for their preparation; a mixture of *ca.* equal amounts of the two preparations had mp 157–157.5°.

Studies on Reactions of Isoprenoids. XII.[1] The 1,4-Cycloaddition Reactions of Nitrosobenzene with Isoprene, Chloroprene, and Myrcene

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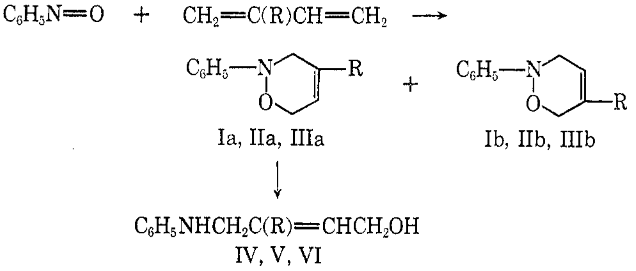
Although 1,4-cycloaddition reactions of nitrosobenzene with 1-mono- and 1,4-disubstituted butadienes have been extensively studied,[2] those with 2-substituted butadienes seem to be still ambiguous with respect to the orientation; isoprene, 2-methoxybutadiene, and chloroprene have been known to react with nitrosobenzene to afford only one major cycloadduct, but the direction of the addition has not been adequately established or shown.[3] In a continuation of our study on the reactivity of myrcene, 7-methyl-3-methylen-1,6-octadiene, in 1,4-cycloaddition reactions, the reaction with nitrosobenzene was investigated in comparison with those of isoprene and chloroprene.[4]

The results are summarized in Table I. In the reaction with isoprene at 20°, only one product (Ia) was isolated as an oil. The direction of the addition was established by reduction of Ia with zinc powder and

TABLE I

PRODUCTS OF THE 1,4 CYCLOADDITIONS OF NITROSOBENZENE WITH ISOPRENE, CHLOROPRENE, AND MYRCENE[a]



R = CH_3 for Ia, Ib, and IV
R = Cl for IIa, IIb, and IV
R = $CH_2CH_2CH{=}C(CH_3)_2$ for IIIa and IIIb, and VI

1,3-Dienes	Reaction temp, °C	Reaction time, hr	Products (yield, %)
Isoprene	20	72	Ia (42
Chloroprene	20	72	IIa (58)
Myrcene	20	72	IIIa (30) + IIIb (16)
Myrcene	80	1	IIIa (3) + IIIb (28)
Myrcene	0–5	24	IIIa (28) + IIIb (8)

[a] All reactions were carried out in benzene.

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[1] Part XI of this series: T. Sasaki, S. Eguchi, and T. Oyobe, *Bull. Chem. Soc. Jap.*, **43**, 1252 (1970).

[2] G. Kresze and J. Firl, *Tetrahedron*, **24**, 1043 (1968), and references cited therein.

[3] J. Hamer and M. Ahmad, "1,4-Cycloaddition Reactions," J. Hamer, Ed., Academic Press, New York, N. Y., 1967, p 427.

[4] T. Sasaki, S. Eguchi, and T. Ishii, *J. Org. Chem.*, **34**, 3749 (1969).

acetic acid to 4-phenylamino-3-methyl-2-buten-1-ol (IV) which structure was confirmed by the nmr data (Table II). On addition of trifluoroacetic acid, the signals at τ 6.25 and 6.79 shifted to lower field of τ 6.20 and 5.41, respectively, while all other signals were not much affected, supporting above assignment. Thus, the structure of Ia was characterized as 2-phenyl-4-methyl-3,6-dihydro-1,2-oxazine. Since no trace of isomeric Ib could be isolated, the 1,4 cycloaddition of nitrosobenzene with isoprene at 20° is concluded to be regiospecific.

Table II

Nmr Data of the 1,4 Cycloadducts and Their Reduction Products[a]

Compd	Chemical shift (J)[b]		Assignment[c]
Ia	2.80 (m)		Phenyl
	4.37 (m)		C-5 vinyl
	5.37 (m)		C-6 methylene
	6.37 (s, br)		C-3 methylene
	8.23 (s, br)		C-4 allylic methyl
IIa	2.50–3.15 (m)		Phenyl
	4.01 (m)		C-5 vinyl
	5.53 (q, 2.2)		C-6 methylene
	6.12 (q, 2.2)		C-3 methylene
IIIa	2.97 (m)		Phenyl
	4.50 (s, br)		C-5 vinyl
	4.92 (m)		Vinyl of the side chain
	5.64 (m)		C-6 methylene
	6.40 (m)		C-3 methylene
	7.94 (s, unsym)		Two methylenes of the side chain
	8.33, 8.40 (s)		*gem*-Dimethyls of the side chain
IIIb	2.95 (m)		Phenyl
	4.47 (s, br)		C-4 vinyl
	4.92 (m)		Vinyl of the side chain
	5.62 (m)		C-6 methylene
	6.39 (m)		C-3 methylene
	7.93 (s, unsym)		Two methylenes of the side chain
	8.33, 8.40 (s)		*gem*-Dimethyls of the side chain
IV	2.60–3.53 (m)		Phenyl
	4.35 (t, 6.0)		C-2 vinyl
	5.77 (d, 6.0)		C-1 methylene
	6.25 (s)	6.20[e]	C-4 methylene
	6.79 (s)[d]	5.41[e]	OH and NH
	8.17 (s)		C-3 allylic methyl
V	2.60–3.53 (m)		Phenyl
	4.08 (t, 7.5)		C-2 vinyl
	5.86 (d, 7.5)		C-1 methylene
	6.06 (s)	5.83[e]	C-4 methylene
	6.78 (s)[d]	3.67[e]	OH and NH
VI	2.87 (m)		Phenyl
	4.23 (t, 6.0)		C-2 vinyl
	4.88 (m)		Vinyl of the side chain
	5.74 (d, 6.0)		C-1 methylene
	6.22 (s)	6.16[e]	C-4 methylene
	6.94 (s)[d]	3.75[e]	OH and NH
	7.90 (s)		Two methylenes of the side chain
	8.30, 8.39 (s)		*gem*-Dimethyls of the side chain

[a] All spectra were run at 60 MHz. Ia in CCl_3 and other compounds in $CDCl_3$. [b] Shifts are reported as τ values, with TMS as an internal reference. [c] All areas are consistent with the assignments. [d] Disappeared on deuteration. [e] Chemical shifts on addition of trifluoroacetic acid. Other signals were not much affected.

In the reaction with chloroprene under the similar conditions, an oily product (IIa) was obtained, which was characterized as 2-phenyl-4-chloro-3,6-dihydro-1,2-oxazine on the basis of its nmr data (Table II) and the reduction product (V). In the nmr spectrum of V, the signal change (τ 6.06 → 5.83, 6.78 → 3.67) on addition of trifluoroacetic acid was also observed as in that of IV. Thus, V was assigned as 4-phenylamino-3-chloro-2-buten-1-ol.

The reaction with myrcene under the same conditions afforded two isomeric oily adducts, IIIa and IIIb, in 30 and 16% yield, respectively (Table I). Both IIIa and IIIb had a molecular formula corresponding to a 1:1 adduct, $C_{16}H_{21}NO$, and m/e 243 (M^+); ultraviolet absorptions $\lambda_{\max}^{\mathrm{EtOH}}$ 204 nm (ϵ 23,800) and 244 (10,400) for IIIa, and 206 (24,200) and 244 (10,200) for IIIb suggested the presence of 3,6-dihydro-1,2-oxazine chromophore.[5] Nmr data (Table II) were very similar to each other, both being compatible with the 1,4 cycloadducts IIIa or IIIb. The direction of the addition was determined by reduction of IIIa to 4-phenylamino-3-(4-methyl-3-pentenyl)-2-buten-1-ol (VI), the structure of which was assigned on the basis of analytical and nmr data: in the nmr spectrum (Table II), the signals at τ 6.22 and 6.94 shifted to τ 6.16 and 3.75, respectively on addition of trifluoroacetic acid, supporting above structural assignment. From this fact, IIIa was finally characterized as 2-phenyl-4-(4-methyl-3-pentenyl)-3,6-dihydro-1,2-oxazine and, therefore, IIIb as its isomer, 2-phenyl-5-(4-methyl-3-pentenyl)-3,6-dihydro-1,2-oxazine.

As shown in Table I, the total and relative yields of IIIa and IIIb were much affected by the reaction temperature. IIIb is less sterically hindered isomer and is thermodynamically more stable than IIIa, being produced at much higher reaction temperature.

Such a reactivity difference between isoprene or chloroprene and myrcene is apparently ascribable to the presence of a long unsaturated substituent instead of a methyl or a chloro, the former exerting a considerable steric interaction with nitrosobenzene in the 1,4-cycloaddition reaction.

Experimental Section[6]

2-Phenyl-4-methyl-3,6-dihydro-1,2-oxazine (Ia).—To a stirred solution of freshly distilled isoprene (4.6 g, 0.067 mol) in dry benzene (40 ml) was added a solution of nitrosobenzene (3.6 g, 0.033 mol) in dry benzene (40 ml) at 20° and stirring was continued for 3 days. Distillation under reduced pressure afforded 2.5 g (42%) of Ia which was further purified by chromatography on an alumina (Woelm, neutral grade I) column eluting with *n*-hexane: bp 80–81° (0.15 mm) [lit.[7] bp 113–115° (1 mm)];

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[5] P. Scheiner, O. L. Chapman, and J. D. Lassila, *J. Org. Chem.*, **34**, 813 (1969).

[6] See footnote 17 in ref 4.

[7] Y. A. Arbuzov and L. K. Lysanchuk, *Dokl. Akad. Nauk SSSR*, **145**, 319 (1962); *Chem. Abstr.*, **57**, 15104 (1962).

$n^{19}\mathrm{D}$ 1.5685 (lit.[7] $n^{20}\mathrm{D}$ 1.5680); ir (neat) 3025, 2920, 1683, 1603, 1496, 1450, 1380, 853, 755, and 691 cm^{-1}.

4-Phenylamino-3-methyl-2-buten-1-ol (IV).—To a solution of Ia (0.50 g, 0.0029 mol) in ether (10 ml) and acetic acid (25 ml) was added zinc powder (6.0 g, 0.092 g-atom) gradually and the mixture was stirred for 4 days at room temperature. After removal of inorganic solids, the mixture was diluted with water and extracted with ether (five 50-ml portions). Combined ether extracts were neutralized with sodium bicarbonate and dried over sodium sulfate and potassium hydroxide. Removal of ether gave a crude product which was passed through an alumina column with benzene to give IV as an oil (0.13 g, 26%): $n^{18}\mathrm{D}$ 1.5780 (lit.[7] $n^{20}\mathrm{D}$ 1.5765); ir (neat) 3400, 3030, 2924, 1675, 1600, 1500, 1445, 1383, 751, and 690 cm^{-1}.

2-Phenyl-4-chloro-3,6-dihydro-1,2-oxazine (IIa).—Chloroprene (5.9 g, 0.067 mol) in xylene (10 ml) was treated similarly with nitrosobenzene (3.6 g, 0.033 mol) in dry benzene (40 ml). Distillation under reduced pressure afforded IIa (3.8 g, 58%) as an oil: bp 101–105° (0.8 mm) [lit.[8] bp 87–88° (0.5 mm)]; $n^{21}\mathrm{D}$ 1.5766 (lit.[8] $n^{20}\mathrm{D}$ 1.5788); ir (neat) 3060, 2880, 2830, 1662, 1600, 1495, 1455, 1431, 1354, 1278, 1214, 760, and 691 cm^{-1}.

4-Phenylamino-3-chloro-2-buten-1-ol (V).—To a solution of IIa (1.0 g, 0.0051 mol) in ether (10 ml) and acetic acid (10 ml) was added zinc powder (3.0 g, 0.046 g-atom) gradually and the mixture was stirred for 1 day at room temperature. Work-up as above afforded a crude product which was purified by a silica gel column, eluting with benzene–methanol to give analytically pure V as an oil (0.70 g, 71%): $n^{21}\mathrm{D}$ 1.5835; ir (neat) 3400, 3040, 2920, 1645, 1603, 1503, 1432, 1320, 1253, 755, and 691 cm^{-1}.

Anal. Calcd for $C_{10}H_{12}NOCl$: C, 60.76; N, 6.12; H, 7.09. Found: C, 60.77; H, 6.14; N, 7.06.

2-Phenyl-4-(4-methyl-3-pentenyl)-3,6-dihydro-1,2-oxazine (IIIa) and 2-Phenyl-5-(4-methyl-3-pentenyl)-3,6-dihydro-1,2-oxazine (IIIb).—Myrcene (9.2 g, 0.067 mol) was treated with nitrosobenzene (3.6 g, 0.033 mol) similarly. The product was distilled under reduced pressure to give IIIa (2.4 g, 30%) and IIIb (1.3 g, 16%), both of which were further purified on an alumina column eluting with *n*-hexane to give analytically pure samples.

Compound IIIa exhibited the following properties: bp 113–115° (0.3 mm); $n^{19}\mathrm{D}$ 1.5539; ir (neat) 3030, 2924, 1664, 1632, 1600, 1497, 1455, 1379, 1218, 1086, 1033, 756, and 688 cm^{-1}; mass spectrum *m/e* (rel intensity) 243 (35.4), 198 (28.0), 162 (100), 156 (54.2), 144 (55.4), 136 (16.7), 135 (28.1), 121 (29.2), 119 (29.2), 109 (36.5), 107 (99.8), 105 (60.4), 104 (62.5), 93 (85.8), 91 (60.8), 79 (70.8), 78 (50.0), 77 (65.8), 69 (93.3), 67 (60.8), and 55 (52.5).

Anal. Calcd for $C_{16}H_{21}NO$: C, 78.97; H, 8.70; N, 5.76. Found: C, 79.44; H, 8.55; N, 6.09.

IIIb was analyzed as follows: bp 122–124° (0.3 mm); $n^{19}\mathrm{D}$ 1.5520; ir (neat) 3020, 2924, 1673, 1600, 1494, 1451, 1377, 1214, 1082, 1028, 753, and 686 cm^{-1}; mass spectrum *m/e* (rel intensity) 243 (97.5), 198 (22.5), 182 (50.8), 168 (50.0), 156 (44.2), 154 (44.2), 153 (40.8), 149 (41.7), 136 (15.9), 135 (25.8), 107 (57.5), 105 (42.5), 93 (94.2), 77 (100), 73 (91.7), 69 (91.7), 57 (34.2), and 55 (25).

Anal. Calcd for $C_{16}H_{21}NO$: C, 78.97; H, 8.70; N, 5.76. Found: C, 78.97; H, 8.59; N, 5.97.

4-Phenylamino-3-(4-methyl-3-pentenyl)-2-buten-1-ol (VI).—IIIa (1.0 g, 0.0041 mol) in ether (10 ml) and acetic acid (25 ml) was reduced with zinc powder (7.5 g, 0.11 g-atom) at room temperature for 5 days. Work-up as above afforded crude VI which was purified by silica gel and alumina columns successively to give pure VI (0.29 g, 29%) as an oil: $n^{19}\mathrm{D}$ 1.5030; ir (neat) 3380, 2924, 1660, 1603, 1504, 1435, 1375, 746, and 689 cm^{-1}.

Anal. Calcd for $C_{16}H_{23}NO$: C, 78.32; H, 9.45; N, 5.71. Found: C, 78.22; H, 9.22; N, 5.77.

Registry No.—Ia, 26332-63-8; IIa, 26332-64-9; IIIa, 26332-65-0; IIIb, 26332-66-1; IV, 26332-67-2; V, 26332-68-3; VI, 26332-69-4; nitrosobenzene, 586-96-9; isoprene, 78-79-5; chloroprene, 126-99-8; myrcene, 123-35-3.

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Trifluoroacetylation of Amino Acids and Peptides under Neutral Conditions

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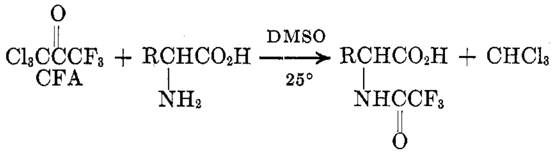
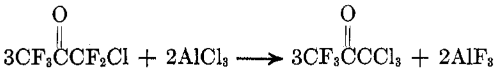
The trifluoroacetyl group has been employed as an alkali-sensitive amino-protecting group[2] for amino acids. Perhaps of more importance, is its use in the preparation of *N*-trifluoroacetylamino acid esters from amino acids derived from natural protein hydrolyzates. These derivatives are volatile and can be analyzed rapidly and quantitatively by gas–liquid chromatography.[3,4] Most recently, rapid chemical and stereochemical analyses of *N*-trifluoroacetylamino acids and peptides using fluorine-19 nuclear magnetic resonance spectroscopy has been reported.[5a]

We have found that several amino acids (see Table I) can be easily *N*-trifluoroacetylated using *sym*-trichlorotrifluoroacetone (CFA) in dimethyl sulfoxide (DMSO). The conditions are mild and essentially neutral. The progress of the reaction can be followed by thin layer chromatography or by gas–liquid chromatography. The latter method gives quantitative results when based on the size of the chloroform peak. CFA[5b] was easily prepared from commercially available chloropentafluoroacetone and aluminum chloride.

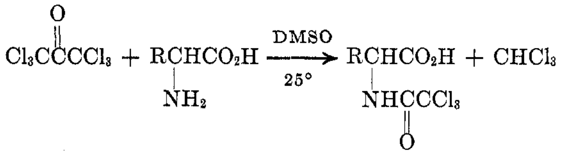


$$\underset{\text{CFA}}{\mathrm{Cl_3C\overset{\overset{\textstyle O}{\|}}{C}CF_3}} + \mathrm{R\underset{\underset{\textstyle NH_2}{|}}{C}HCO_2H} \xrightarrow[25^\circ]{\text{DMSO}} \mathrm{R\underset{\underset{\textstyle NH\underset{\underset{\textstyle O}{\|}}{C}CF_3}{|}}{C}HCO_2H} + \mathrm{CHCl_3}$$



$$\mathrm{3CF_3\overset{\overset{\textstyle O}{\|}}{C}CF_2Cl} + \mathrm{2AlCl_3} \longrightarrow \mathrm{3CF_3\overset{\overset{\textstyle O}{\|}}{C}CCl_3} + \mathrm{2AlF_3}$$

Although the actual mechanism of the reaction was not studied, the products showed that CFA was cleaved between the carbonyl carbon atom and the trichloromethyl moiety. Evidently, the trifluoromethyl group is a much poorer leaving group than is the trichloromethyl portion. This fact was established earlier by comparison of the reactions of hexachloroacetone and hexafluoroacetone with amino acids. The former work,[6] performed in this laboratory, found that *N*-trichloroacetylamino acids (**1**) were the major products.



$$\mathrm{Cl_3C\overset{\overset{\textstyle O}{\|}}{C}CCl_3} + \mathrm{R\underset{\underset{\textstyle NH_2}{|}}{C}HCO_2H} \xrightarrow[25^\circ]{\text{DMSO}} \mathrm{R\underset{\underset{\textstyle NH\underset{\underset{\textstyle O}{\|}}{C}CCl_3}{|}}{C}HCO_2H} + \mathrm{CHCl_3}$$

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