*Robert A. Earl, Fred W. Clough and Leroy B. Townsend**

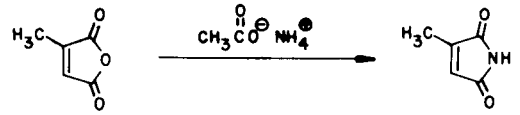
Division of Medicinal Chemistry, Department of Biopharmaceutical Sciences and Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

Received April 25, 1978

The chemical reactivity of citraconimide (3-methylmaleimide, **2**) has been investigated with special emphasis being placed on the use of mild and selective reaction conditions. Particular attention has been focused on the theiotropic reactivity of citraconimide. The results indicate that the reaction of various mercaptans with citraconimide is highly regioselective resulting predominately in the geminally substituted products with the percentage of vicinally substituted products increasing as a function of the steric bulk of the alkyl mercaptan. Additional studies on the double bond of citraconimide have furnished 4-halogeno- and the 3,4-dihydro derivatives of citraconimide. Several *N*-substituted citraconimide derivatives have also been prepared.

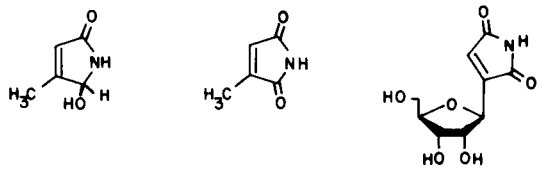
*J. Heterocyclic Chem.*, 15, 1479 (1978)

Jatropham (**1**), isolated from the chloroform extracts of *Jatropha macrorhiza Benth* (2), has been shown to exhibit inhibitory activity toward P-388 lymphocytic leukemia. In addition, various substituted maleimides (3) have demonstrated theiotropic reactivity of the type that is generally accepted as the mechanism of action of a number of naturally occurring biologically active agents including terpenes (4) (*e.g.*, elephantopin) and the nucleoside antibiotic showdomycin (**3**) (5). It was also demonstrated (2) that jatropham (**1**) could be readily converted to citraconimide (**2**) which was of considerable interest



**2**

due to the obvious structural similarities between citraconimide and showdomycin, a nucleoside of current and continuing research interest in our laboratory. This suggested that **2** should be useful as a model for chemical studies on the aglycon portion of showdomycin. The cumulative nature of these facts prompted us to undertake an investigation of various aspects of the chemical reactivity of citraconimide.



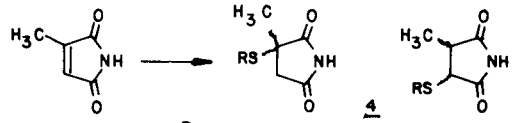
**1** **2** **3**

## Results and Discussion.

It was necessary to have available a sufficient quantity of citraconimide (**2**) in order to successfully conduct this investigation. Although several syntheses of citraconimide (**2**) had been previously reported (6), none of these proved to be useful on a preparative scale in our laboratory. This prompted us to develop an alternative synthesis which involved the reaction of citraconic anhydride with a 0.25 mole excess of ammonium acetate in acetic acid at reflux temperature. After distillation of the crude product, a 35% yield of slightly impure, crystalline citraconimide (**2**) was realized. Recrystallization of this material from benzene or sublimation resulted in a pure homogeneous product.

## Reaction with Alkyl Mercaptans.

Because of the generally accepted theiotropic nature of showdomycin as well as other naturally occurring bioactive agents (*vide supra*), it was of considerable interest to examine the course of the reaction of citraconimide with a variety of alkyl mercaptans. It had been reported (2) that the addition of glutathione to *N*-ethylcitraconimide resulted in a single adduct and that the reaction rate, but not the regioselectivity of the reaction, was markedly influenced by substitution on the double bond. However, these experiments did not examine the influence of the thiophile on the regiochemical course of the reaction. It was therefore decided that the reaction of citraconimide with mercaptans of increasing steric requirements should be examined in order to establish the effect of steric bulk of the thiophile on the regioselectivity of the addition. In the present investigation, mixtures of citraconimide in a *p*H 7.4 buffer were treated at 0° with various alkyl mercaptans (*e.g.*, methyl-, ethyl-, isopropyl- and *t*-butylmercaptan). The crude products (**4**) were isolated by extraction and then purified by short-path distillation. In order to determine the regioselectivity



**4**

| R | | |
|---|---|---|
| $CH_3$- | 94 | 6 |
| $C_2H_5$- | 95 | 5 |
| $(CH_3)_2CH$- | 90 | 10 |
| $(CH_3)_3C$- | 90 | 10 |

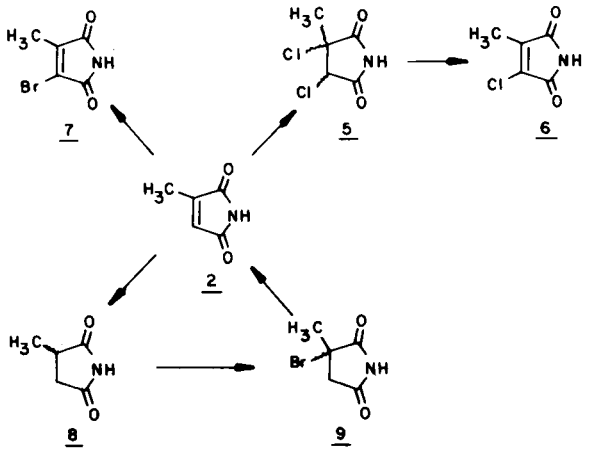
of the addition reactions, the crude reaction products (*i.e.*, after extraction) were also examined by gas-liquid chromatography. It was necessary to silylate the crude product mixture prior to glc examination with *N,O-bis*-trimethylsilylacetamide (BSA) in order to improve the resolution of the chromatogram. The glc experiments, in conjunction with pmr spectral analysis (*vide infra*), established that methylmercaptan and ethylmercaptan had added regioselectively to the double bond of citraconimide to give the adduct with the alkylthio group geminal to the methyl moiety of citraconimide in greater than 94% purity. However, the reaction of citraconimide with isopropylmercaptan and *t*-butylmercaptan were not as regioselective and furnished only 90% of the geminally substituted product. This decrease in regioselectivity is most probably attributable to the increasing steric bulk in proceeding to the isopropyl- and *t*-butylmercaptans.

Pmr spectroscopy substantiated that the major products were as indicated above when the methyl group of the alkylthio-3-methylsuccinimides appeared as a singlet. For example, in the case of **4** (R = methyl), the simplest case in which to decipher the pmr spectrum of the crude reaction mixture, the major product exhibits singlets for the 3-methyl and methylthio moieties at δ 1.54 and δ 2.16, respectively. In addition, the methylene protons appear as an AB-quartet with a coupling constant of 18 Hz. In comparison, the 3-methyl group of the minor product (*i.e.*, the vicinally substituted product) appears as a doublet centered at δ 1.3 and the methylthio protons resonate as a singlet at δ 2.2. The doublet of the 3-methyl moiety in this minor product shows a splitting of 7 Hz which is comparable to that found for the splitting of the methyl group of 3-methylsuccinimide (J = 6 Hz, see Experimental).

Reaction of the Double Bond (Scheme I).

Chlorination of citraconimide (**2**) with chlorine in a mixture of methylene chloride and carbon tetrachloride was complete within two minutes after exposure to bright sunlight. The product was isolated (67% yield) and the structure assigned as *d*,ℓ-3,4-dichloro-3-methylsuccinimide (**5**) by pmr spectroscopy (singlet for the methyl group and the presence of an exchangeable proton) and elemental analysis. Dehydrohalogenation of **5** to afford 3-chlorocitraconimide (**6**) was accomplished in 85% yield by simply heating **5** in water for four hours.

Scheme I



In direct contrast, the reaction of **2** with bromine water was sluggish and required several days before starting material was no longer detectable by tlc and eventually furnished 4-bromocitraconimide (**7**) (7). However, the reaction of **2** with bromine in acetic acid at reflux with concomitant irradiation (heat lamp) resulted in a more rapid disappearance of bromine and production of pure **7** in 28% yield. The yield was slightly improved (up to 45%) when the reaction was carried out in acetic anhydride employing an excess of bromine. The fact that *d*,ℓ-3,4-dibromo-3-methylsuccinimide was not isolated in this reaction, in analogy to the chlorination experiment above, may simply be a consequence of the difference in the reaction conditions (*i.e.*, either temperature or the presence of sodium acetate); however, these parameters were not studied further. Parenthetically, 3-bromo-*d*,ℓ-citraconimide (**7**) demonstrated growth inhibitory activity against cultured HeLa cells (8) comparable to that of showdomycin.

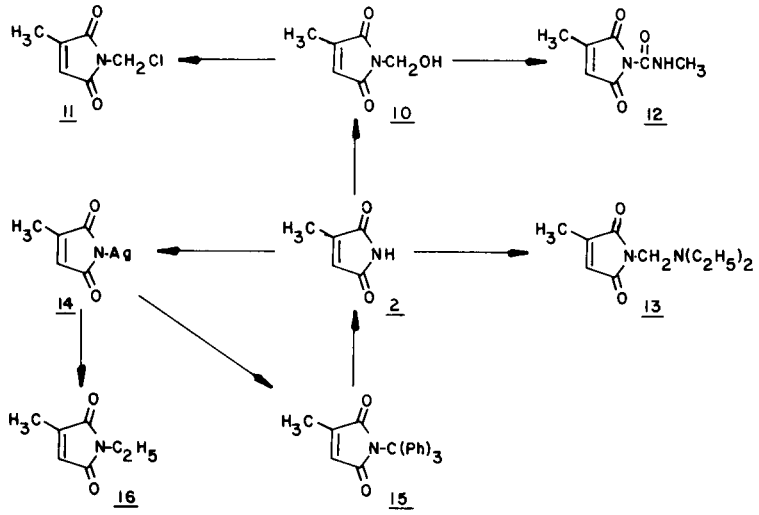
Citraconimide (**2**) is readily reduced catalytically (Pd-C/$H_2$) to *d*,ℓ-3-methylsuccinimide (**8**) which, when treated with one equivalent of *N*-bromosuccinimide in refluxing carbon tetrachloride and irradiated with a heat lamp resulted in a single product which analyzed as a monobromo derivative. The pmr spectrum revealed the presence of an exchangeable proton (therefore not an *N*-bromo compound), a methyl group (singlet) and a methylene group (AB-quartet) which indicated that the product must have the structure **9** with the newly introduced bromine atom being geminal to the methyl group. Heating **9** in water resulted in dehydrohalogenation and regeneration of citraconimide (**2**) in 53% yield.

Reactions of the Imide Function (Scheme II).

Citraconimide (**2**) reacted with formalin (9) at *p*H 5 to yield *N*-hydroxymethylcitraconimide (**10**). Reaction of **10** with phosphorus trichloride in dry acetone produced *N*-chloromethylcitraconimide (**11**). Furthermore, the reaction of **2** with methylisocyanate (10) in dry dimethyl sulfoxide at ambient temperature yielded *N*-(*N'*-methylcarbamoyl)citraconimide (**12**). Similarly, **2** reacts with diethylaminoethyl ethyl ether (9,11) at 80° to give *N*-(*N'*,*N'*-diethylaminomethyl)citraconimide (**13**).

Another route, employing the silver salt of citraconimide (**2**) was also found to be applicable for modification of the imide function. In conjunction with this line of

Scheme II




investigation, it was found that the silver salt **14** could conveniently be prepared by simply stirring citraconimide (**2**) with silver oxide in dry acetonitrile at room temperature with the exclusion of light. The silver salt **14**, without further characterization, was reacted with trityl chloride in toluene at reflux or iodoethane in benzene at reflux to give *N*-tritylcitraconimide (**15**) and *N*-ethylcitraconimide (**16**) (2), respectively.

Finally, it was of interest to investigate the feasibility of regenerating citraconimide from **15**. Indeed, it was found that by dissolving **15** in trifluoroacetic acid containing three equivalents of water, a bright yellow-red solution was immediately formed, with the resultant formation of citraconimide (**2**) and triphenylcarbinol. This indicates that tritylation could be useful in masking the imide function, if necessary, and subsequent reconstitution would still be possible under mild conditions.

## EXPERIMENTAL (12)

Citraconimide (**2**).

Ammonium acetate (224 g., 2.9 moles, predried at 70° for 1 hour) and citraconic anhydride (224 g., 2.0 moles) were added to 400 ml. of glacial acetic acid and the mixture slowly heated to reflux. After heating at reflux for 2 hours, the solution was cooled to ambient temperature and evaporated *in vacuo* at 70°. The dark syrupy residue was extracted with boiling ethyl acetate (8 x 200 ml.) and the extracts were concentrated to dryness to give a yellow solid. This solid was then distilled and the fraction boiling at 122-125° (30 mm) collected. The distillate crystallized on cooling and was recrystallized from benzene to give 64.22 g. of **2** (29%), m.p. 103.5-105.5° (Lit. (6) 108°); $^1$H nmr (DMSO-$d_6$): δ 2.0 (d, 3, $J_{CH_3,H\text{-}4}$ = 2 Hz), 6.56 (d, 1, $J_{CH_3,H\text{-}4}$ = 2 Hz), 10.78 (bs. 1, NH, exchangeable).

General Procedure for the Reaction of Citraconimide with Alkyl Mercaptans.

A solution of citraconimide (0.55 g., 5.0 mmoles) in 25 ml. of a *p*H 7.4 buffer (disodium phosphate/citric acid) was cooled to 0° in an ice bath and then 13.7 mmoles of the desired mercaptan was added *via* a syringe. The reaction mixture was allowed to stir for 0.5 hour at 0° and was then concentrated *in vacuo* to 5 ml. This solution was then extracted with ethyl acetate (5 x 12 ml.), the combined extracts dried (sodium sulfate), evaporated *in vacuo* to a syrup and then purified by short-path distillation.

Compound **4**, R = Methyl.

Distillation (140°, 0.5 mm) yielded 0.7 g. (88%) of a clear liquid; $^1$H nmr (DMSO-$d_6$): δ 1.55 (s, 3, $CH_3$), 2.15 (s, 3, $CH_3$S-), 2.63 and 2.99 (AB-quartet, 2, H-4 J = 18 Hz), 8.28 (bs, 1, NH, exchangeable).

*Anal.* Calcd. for $C_6H_9NO_2S$: C, 45.26; H, 5.69; N, 8.79. Found: C, 44.99; H, 5.73; N, 8.54.

Compound **4**, R = Ethyl.

Distillation (90°, 0.5 mm) gave 0.79 g. (91%) of a clear liquid. $^1$H nmr (DMSO-$d_6$): δ 1.23 (t, 3, J = 8 Hz, -$CH_2CH_3$), 1.67 (s, 3, $CH_3$), 2.8 (q, 2, J = 8 Hz, -$CH_2CH_3$), 2.83 (s, 2, $CH_2$), 8.12 (bs, 1, NH, exchangeable).

*Anal.* Calcd. for $C_7H_{11}NO_2S$: C, 48.53; H, 6.40; N, 8.08. Found: C, 48.45; H, 6.39; N, 8.00.

Compound **4**, R = Isopropyl.

Distillation (125°, 0.1 mm) resulted in 0.9 g. (96%) of a clear liquid; $^1$H nmr (deuteriochloroform): δ 1.37 (d, 6, $(CH_3)_2$-CH, J = 4.5 Hz), 1.7 (s, 3, $CH_3$), 2.83 (s, 2, $CH_2$), 3.33 (m, 1, $(CH_3)_2CH$, 10.16 (bs, 1, NH).

*Anal.* Calcd. for $C_8H_{13}NO_2S$: C, 51.33; H, 7.00; N, 7.48. Found: C, 51.35; H, 6.94; N, 7.16.

Compound **4**, R = *t*-Butyl.

In this case, the reaction mixture was stirred for 24 hours before work-up. Distillation (135°, 0.1 mm) resulted in 0.6 g. (59%) of a clear liquid; $^1$H nmr (DMSO-$d_6$): δ 1.37 (s, 3, $CH_3$), 1.62 (s, 9, $C(CH_3)_3$), 2.88 (s, 1), 2.96 (s, 1), 11.33 (bs, 1, NH, exchangeable).

*Anal.* Calcd. for $C_9H_{15}NO_2S$: C, 53.72; H, 7.51; N, 6.96. Found: C, 53.54; H, 7.84; N, 6.93.

*d,l*-3,4-Dichloro-3-methylsuccinimide (**5**).

Small portions of a solution of chlorine in carbon tetrachloride

were added slowly to citraconimide (1.11 g., 0.01 mole) dissolved in 50 ml. of dry methylene chloride. After each addition, the reaction mixture was exposed to bright sunlight to decolorize the solution. When all the citraconimide had been consumed, as determined by tlc (heptane/ethyl acetate; 6:4), the reaction mixture was evaporated to a syrup which crystallized on cooling. The crude reaction product was recrystallized from benzene/cyclohexane and then sublimed to give 1.39 g. (76%) of **5** as a white solid, m.p. 93-94.5°; $^1$H nmr (deuteriochloroform): δ 1.9 (s, 3, $CH_3$), 4.89 (s, 1, H-4), 8.95 (bs, 1, NH).

*Anal.* Calcd. for $C_5H_5Cl_2NO_2$: N, 7.69. Found: N, 8.04.

3-Chlorocitraconimide (**6**).

A heterogeneous mixture of **5** (1.5 g., 0.098 mole) in 15 ml. of water was heated on a steam bath for 4 hours and then allowed to cool at room temperature overnight. The precipitate was filtered to give 0.98 g. (85%) of **6**, m.p. 147-148.5°; $^1$H nmr (DMSO-$d_6$): δ 1.96 (s, 3, $CH_3$), 8.5 (bs, 1, NH).

*Anal.* Calcd. for $C_5H_4ClNO_2$: C, 42.70; H, 2.86; N, 9.64. Found: C, 43.05; H, 2.86; N, 9.44.

3-Bromocitraconimide (**7**).

To a cooled (ice bath), magnetically stirred solution of citraconimide (0.55 g., 4.9 mmoles) and 0.41 g. of anhydrous sodium acetate in 5 ml. of glacial acetic acid was added dropwise 6.2 ml. of a solution of bromine in acetic acid (1.29 g. of bromine/10 ml. of acetic acid). The reaction mixture was heated to reflux and irradiation with a heat lamp resulted in the disappearance of the characteristic bromine color and precipitation of a solid. The precipitate was removed by filtration and the filtrate was evaporated to dryness to furnish a solid material. The solid was extracted with chloroform (3 x 15 ml.), filtered and concentrated to dryness to give the product. Recrystallization first from benzene and then from water yielded 0.42 g. (45%) of **7**, m.p. 175-178° (lit. (7) 176°); $^1$H nmr (DMSO-$d_6$): δ 1.83 (s, 3, $CH_3$), 11.5 (bs, 1, NH).

*Anal.* Calcd. for $C_5H_4BrNO_2$: C, 31.60; H, 2.16; N, 7.36. Found: C, 31.89; H, 2.19; N, 7.33.

3-Methylsuccinimide (**8**).

A suspension of citraconimide (2.0 g., 18 mmoles) and 5% palladium on charcoal (0.5 g.) in 50 ml. of water was hydrogenated at atmospheric pressure until the theoretical amount (436 ml.) of hydrogen had been consumed. The catalyst was removed by filtration, the filtrate evaporated to dryness *in vacuo* and the residue was recrystallized from benzene to give 1.27 g. (62.4%) of **8**. This material was sublimed (60°, 0.5 mm) to give an analytical sample which melted at 66-68.5°; $^1$H nmr (DMSO-$d_6$): δ 1.23 (d, 3, $CH_3$, J = 6 Hz), 2.0-3.1 (m, 3), 11.1 (bs, 1, NH).

*Anal.* Calcd. for $C_5H_7NO_2$: C, 53.09; H, 6.24; N, 12.38. Found: C, 53.27; H, 6.12; N, 12.43.

*d*,ℓ-3-Bromo-3-methylsuccinimide (**9**).

To a suspension of **8** (0.91 g., 8.0 mmoles) and *N*-bromosuccinimide (1.62 g., 9.1 mmoles) in 70 ml. of carbon tetrachloride was added 20 mg. of 2,2′-azobis-(2-methylpropionitrile). The reaction mixture was protected with a calcium chloride drying tube and heated at reflux for 2 hours. An additional 20 mg. of the radical initiator was then added and heating continued for 20 hours (the final 2 hours with concomitant irradiation by heat lamp). After cooling to room temperature, the reaction mixture was evaporated to dryness and the residue chromatographed on a SilicAR CC-7 column (1.5 cm x 15 cm) with the collection of 15 ml. fractions. Evaporation of the appropriate fractions [3-7, as evidenced by tlc, hexane-ethyl acetate 4:1 (v/v)] to dryness provided a residue which was recrystallized from benzene (or cyclohexane) to give 0.83 g. (54%) of **9**, m.p. 92-93.5°; $^1$H nmr (DMSO-$d_6$): δ 1.72 (s, 3, $CH_3$), 3.18 and 3.16 (AB-quartet, 2, H-4, J = 19 Hz), 7.5 (bs, 1, NH).

*Anal.* Calcd. for $C_5H_6BrNO_2$: C, 31.27; H, 3.15; N, 7.29. Found: C, 30.94; H, 3.18; N, 7.28.

Regeneration of Citraconimide from **9**.

A mixture of **9** (0.05 g., 0.26 mmole) in 3 ml. of water was heated on a steam bath overnight (18 hours). The solution was evaporated under a stream of air to dryness and the residue recrystallized from benzene to give 15 mg. (52%) of citraconimide (identified by m.p., mixed m.p. and spectral comparison with an authentic sample).

*N*-Hydroxymethylcitraconimide (**10**).

Sodium hydroxide (1.25*N*, about 0.4 ml.) was added to a mixture of citraconimide (7.75 g., 0.07 mole) and 5.9 ml. of 37% formaldehyde solution so that the *p*H was 5. The mixture was gently warmed on a steam bath to effect solution and then allowed to stand at room temperature for 2 days. Since tlc indicated that the reaction was incomplete, another 1 ml. of formaldehyde solution was added and the mixture heated on a steam bath. After 1 hour, tlc revealed the absence of starting material and the reaction mixture was evaporated to dryness *in vacuo*. The resulting residue was dissolved in benzene, dried (sodium sulfate), concentrated to 15 ml. and the product precipitated by the addition of 60 ml. of heptane. Recrystallization from diethyl ether gave 9.15 g. (92%) of **10** as white crystals, m.p. 60-64°; $^1$H nmr (DMSO-$d_6$): δ 1.97 (s, 3, $CH_3$), 4.73 (d, 2, J = 6 Hz, -$CH_2$OH), 6.15 (t, 1, J = 6 Hz, -$CH_2O$*H* exchangeable), 6.6 (s, 1, H-4).

*Anal.* Calcd. for $C_6H_7NO_3$: C, 51.07; H, 5.00; N, 9.92. Found: C, 51.19; H, 4.73; N, 9.76.

*N*-Chloromethylcitraconimide (**11**).

Phosphorus trichloride (0.6 ml.) was added dropwise to a cooled (0°) solution of **10** (2.5 g., 17.7 mmoles) in 30 ml. of dry acetone with continuous stirring. After warming to ambient temperature, the reaction was complete within 1 hour as indicated by tlc. The reaction mixture was evaporated to dryness *in vacuo* (30°), the residue was dissolved in 50 ml. of ethyl acetate and the solution extracted with water (5 x 15 ml.), dried (sodium sulfate) and evaporated *in vacuo* to a syrup. Trituration with isopropyl ether (30 ml.) gave a crystalline mass containing some syrupy material. The solid was collected by filtration and sublimed (50°, 0.5 mm) to give 1.38 g. (48%) of **11** as white crystals, m.p. 48-49°; $^1$H nmr (DMSO-$d_6$): δ 2.05 (d, 3, $J_{CH_3,H\text{-}4}$ = 2 Hz), 5.4 (s, 2, $CH_2$Cl), 6.85 (d, 1, H-4, $J_{CH_3,H\text{-}4}$ = 2 Hz).

*Anal.* Calcd. for $C_6H_6ClNO_2$: C, 45.16; H, 3.79; N, 8.77. Found: C, 45.19; H, 3.67; N, 8.71.

*N*-(*N*′-Methylcarbamoyl)citraconimide (**12**).

Methylisocyanate (1.25 g., 22 mmoles) was added to a solution of citraconimide (1.0 g., 9.0 mmoles) in 20 ml. of dry dimethylsulfoxide. The flask was sealed and the solution stirred at room temperature. After 22 hours, tlc indicated that the reaction was complete. The reaction mixture was evaporated to dryness and the resulting pink solid was recrystallized from absolute ethanol (charcoal) to give 0.92 g. (61%) of **12** as a white solid, m.p. 145-147°; $^1$H nmr (deuteriochloroform): δ 2.2 (d, 3, $CH_3$, $J_{CH_3,H\text{-}4}$ = 2 Hz), 2.97 (d, 3, N-$CH_3$, J = 6 Hz), 6.47 (m, 1, H-4), 7.3 (bs, 1, NH).

*Anal.* Calcd. for $C_7H_8N_2O_3$: C, 50.00; H, 4.79; N, 16.66. Found: C, 50.02; H, 4.81; N, 16.77.

*N*-(*N'*,*N'*-Diethylaminomethyl)citraconimide (**13**).

Diethylaminomethyl ethyl ether (9,11) (1.35 g., 10.3 mmoles) was added to citraconimide (1.0 g., 9.0 mmoles). The reaction mixture turned yellow immediately and most of the citraconimide had dissolved. The reaction mixture was then heated to 80° (bath temperature) over a period of 0.5 hour and maintained at that temperature for 10 minutes. Cooling to room temperature and evaporation *in vacuo*, and distillation (78-80°, 1 mm) gave 1.45 g. (82%) of **13** as a yellow liquid; $^1$H nmr (deuteriochloroform): δ 1.2 (t, 6, $CH_3$, J = 6 Hz), 2.17 (d, 3, $CH_3$, $J_{CH_3,H\text{-}4}$ = 2 Hz), 2.63 (q, 4, $CH_2$, J = 6 Hz), 4.5 (s, 2, $CH_2$), 6.37 (m, 1, H-4, J = 2 Hz).

*Anal.* Calcd. for $C_{10}H_{16}N_2O_2$: C, 61.20; H, 8.22; N, 14.27. Found: C, 61.19; H, 8.24; N, 14.41.

Silver Salt of Citraconimide (**14**).

A mixture of citraconimide (3.33 g., 0.03 mole) and dry silver oxide (3.45 g., 0.015 mole) in 60 ml. of dry acetonitrile was stirred at room temperature overnight in the dark. The black silver oxide had been replaced by a white-grey precipitate. The reaction mixture was cooled in an ice bath and the solid removed by filtration. The product was then washed with cold acetonitrile (30 ml.) and dried (2 hours at room temperature *in vacuo*) to give 5.59 g. (86%) of a product which was used without further purification for subsequent reactions.

*N*-Tritylcitraconimide (**15**).

The dry compound **14** (5.4 g., 24.8 mmoles) and freshly recrystallized and dried trityl chloride (6.9 g., 24.8 mmoles) were added to 40 ml. of dry toluene, the mixture was heated at reflux for 3 hours and then stirred at room temperature overnight (18 hours). The reaction mixture was filtered, evaporated to dryness and the resulting residue extracted with boiling chloroform (3 x 30 ml.). The chloroform extracts were evaporated *in vacuo* to 20 ml. and on cooling, the product crystallized. Recrystallization from a mixture of toluene/petroleum ether gave 4.4 g. (50%) of pure **15**, m.p. 153-154°; $^1$H nmr (DMSO-$d_6$): δ 1.9 (s, 3, $CH_3$), 6.47 (s, 1, H-4), 7.3 (m, 15, C-$Ph_3$).

*Anal.* Calcd. for $C_{24}H_{19}NO_2$: C, 81.56; H, 5.42; N, 3.96. Found: C, 81.60; H, 5.54; N, 3.68.

*N*-Ethylcitraconimide (**16**).

In a similar fashion, compound **14** (2.9 g., 13.2 mmoles) and iodoethane (3.12 g., 20 mmoles) were heated at reflux in 40 ml. of dry benzene for 18 hours. The mixture was cooled to room temperature, filtered, the solvent was evaporated *in vacuo* and the residue distilled (101°, 20 mm; Lit. (3) 93°, 13 mm) to give 1.1 g. (80%) of product; $^1$H nmr (deuteriochloroform): δ 1.17 (t, 3, $CH_2CH_3$, J = 6 Hz), 2.1 (s, 3, $CH_3$), 3.5 (d, 2, $CH_2CH_3$, J = 6 Hz), 6.37 (s, 1, H-4).

Regeneration of Citraconimide from **15**.

Compound **15** (353 mg., 1.4 mmoles) was added to a mixture of trifluoroacetic acid (3 ml.) and water (0.1 ml., 3 equivalents) and the mixture was stirred at room temperature for 3 hours. The reaction mixture immediately turned a deep yellow-red and remained that color throughout the reaction. The solvents were evaporated and the residue was purified by preparative tlc on silica gel plates ($R_f$ = 0.67; chloroform/methanol [95/5; v/v]) to yield 0.12 g. (80%) of citraconimide which was identical in physical (m.p., mixed m.p.) and spectral characteristics with an authentic sample.

Acknowledgement.

Support for this research by the Public Health Service Research Grant No. CA-14252, awarded by the National Cancer Institute, Department of Health, Education and Welfare is gratefully acknowledged.

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(1) A preliminary account of this work was presented at the Utah Academy of Sciences, Arts and Letters, Fall Meeting, Provo, Utah, 1976.

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(6) See K. Kojima, N. Voda and C. S. Marvel, *J. Polym. Sci.*, Pt. A-1, 4, 1121 (1966) and references therein.

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(8) Cytotoxicity testing was performed by Shionogi & Co., Ltd., Sanchome, Doshomachi, Osaka, Japan. The HeLa cells used in this experiment were grown in Eagle's minimum essential medium containing 10% bovine serum. For subcultivation, cells were removed from the glass with 0.03% trypsin and 0.04% ethylene diamine tetra-acetic acid. The cells were then resuspended in fresh medium, diluted to a concentration of $10^5$ cells/ml. and implanted in a series of triplicate culture tubes. Drugs were added to the cell cultures on the second day of cultivation. After two days of incubation with a drug, the cell population was measured by electronic cell counter. The effective dose for 50% inhibition ($ED_{50}$) was determined by plotting the logarithmic curve of drug concentration against growth rate (number of treated cells/number of control cells).

| Sample | 40 μg./ml. | 20 μg./ml. | 10 μg./ml. | 5 μg./ml. | (μg./ml.; $ED_{50}$) |
|---|---|---|---|---|---|
| **3** | -- | 72.4 | 13.7 | 10.4 | 15 |
| **2** | 63.5 | 9.3 | 4.6 | -4.8 | 34 |
| **8** | 9.8 | -1.5 | -1.2 | -5.8 | -- |
| **7** | 134.6 | 91.5 | 28.2 | -2.5 | 13 |
| **11** | 127.0 | 57.5 | 15.7 | 10.5 | 18 |
| **10** | 133.7 | 51.2 | 8.3 | -5.4 | 20 |

(9) For similar reactions with maleimide, see P. O. Tawney, R. H. Snyder, R. P. Conger, K. A. Leibbrand, C. H. Stiteler and A. R. Williams, *J. Org. Chem.*, 26, 151 (1961).

(10) For the reaction of methylisocyanate with uracil derivatives see R. Parthasarathy, J. Ohrt, S. P. Dutta and G. B. Chheda, *J. Am. Chem. Soc.*, 95, 8141 (1973).

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(12) All melting points were taken on a Thomas-Hoover apparatus and are uncorrected. All evaporations were done at a temperature ≤ 35° unless noted otherwise. $^1$H nmr chemical shifts are reported relative to DSS (DMSO-$d_6$) or TMS (deuteriochloroform) as an internal reference and were recorded on either a Jeol C60h spectrometer or an EM-390 MHz spectrometer. Gas liquid chromatographic analyses were done with a F & M Model 700 Series gas chromatograph equipped with a thermal conductivity detector. A 4 ft. OV 17 column operating with an inlet temperature of 268°, column temperature of 151°, detector temperature of 262° and a flow rate of 4 ml./sec. separated the products very efficiently.