cold 10% potassium carbonate solution and 25 ml. of saturated brine. The ether solution was dried over magnesium sulfate and evaporated on a water bath at 50°. The residue was dissolved in 100 ml. of petroleum ether and crystallized at Dry Ice-acetone bath temperatures. The product was recrystallized from petroleum ether to give 2.90 g. (35%) of glistening plates, m.p. 74.0–74.5° (reported[7] m.p. 74.5–75.5°).

Method D. N-Nitro-N-methylaniline. To a stirred solution of 1.50 g. (0.016 mole) of aniline in 50 ml. dry ether at 0° was added alternatively every 60 seconds 20 ml. (0.019 mole) of 0.94*M* phenyllithium in ether and 2.20 ml. (0.016 mole) of amyl nitrate until 60 ml. in all of phenyllithium solution and 6.60 ml. of amyl nitrate (three portions of each) had been added. The mixture was stirred 15 min., washed once with 50 ml. of water and twice with 25 ml. of 2*M* potassium hydroxide solution. The combined aqueous solutions were saturated with carbon dioxide. Then 28 g. (0.33 mole) of sodium bicarbonate and 15 ml. (0.16 mole) of methyl sulfate was added, and the mixture was stirred 5 hr. after which another portion (5 ml.) of methyl sulfate was added, and stirring was continued for 12 hr. Sufficient water was added to dissolve the sodium bicarbonate, and the solution was extracted two times with 50-ml. portions of ether. The combined ether extracts were washed twice with 10-ml. portions of 2*M* potassium hydroxide solution, dried over magnesium sulfate, and evaporated on a 50° water bath. The residue was dissolved in 50 ml. of dry ether and chromatographed on a 1.5 × 15 cm. column of activity grade I alumina using ether as eluant. The product was rapidly eluted. The residue from evaporation of the ether was crystallized from petroleum ether to give 1.28 g. (52%) of glistening white plates, m.p. 36.6–37.6° (reported[8] m.p. 38.5–39.5°).

The solution in which the reaction was carried out was extracted three times with 20 ml. of 5% hydrochloric acid. The combined acid solutions were made just basic and shaken with 5 ml. of acetic anhydride until the acrid odor of the latter disappeared. The acetanilide (0.40 g. (18%), m.p. 112°) resulting was isolated by ether extraction.

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(8) E. Bamberger, *Ber.*, **27**, 367 (1894).

Reactions with Diazoalkanes. I. Action of Diazomethane on *N*-Phenylmaleimide, with a Special Note on the Pyrolysis Products of the Pyrazoline

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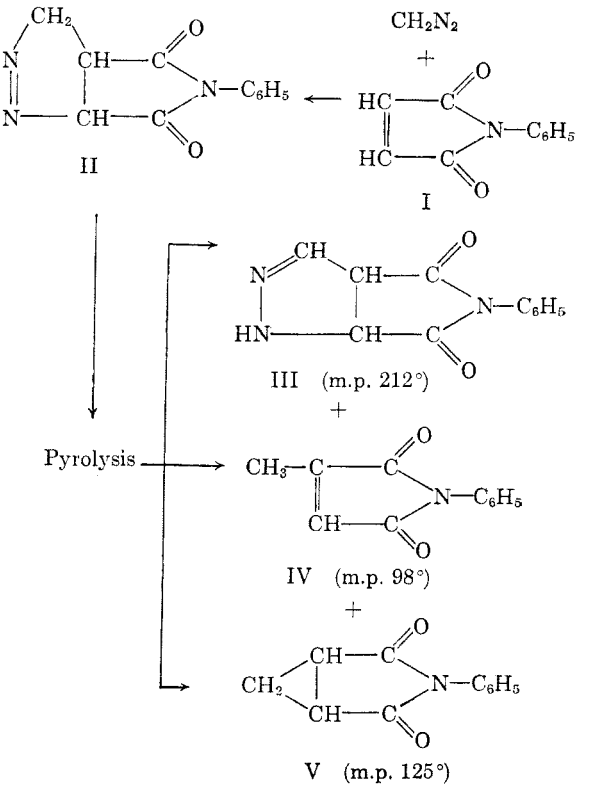
Recently Mustafa *et al.*[1] claimed that diazomethane reacts with *N*-phenylmaleimide (I) in the cold to yield directly Δ^2-pyrazoline (III). They also claimed that pyrolysis of III gave only one product, m.p. 98°, which they described as cyclopropane-2,3-(*N*-phenyl)dicarboximide from its identity with Perkin's product.[2]

(1) A Mustafa, S. M. A. Zayed, and S. Khattab, *J. Am. Chem. Soc.*, **78**, 145 (1956).

We have reinvestigated the action of diazomethane on *N*-phenylmaleimide and found that the product was a derivative of Δ^1-pyrazoline and not of Δ^2-pyrazoline (III) as claimed by Mustafa *et al.*[1]

This conclusion was based on the absence of any N—H stretching frequency in its infrared spectrum.[3]



Pyrolysis of the Δ^1-pyrazoline derivative (II). On heating the Δ^1-pyrazoline derivative (II) at 130° [*cf.* ref. (1)], it was recovered unchanged. However, when the substance was heated above its melting point in a vacuum for about six hours, it gave a mixture of three substances melting at 98°, 125°, and 212° (*cf.* Experimental). The main product, m.p. 98°, described by Mustafa *et al.*[1] to be a cyclopropane derivative (V), proved to be *N*-phenylcitraconimide by identity with an authentic specimen. Its ultraviolet spectrum[4] showed two bands at λ_{max} 223, 275 mμ, ϵ_{max} 18,300, 2600, respectively (Fig. 1), and was very similar to that of maleimide λ_{max} 220, 275 mμ, ϵ_{max} 9,300, 3,000, respectively (*cf.* Fig. 1). The slight bathochromic shift and the hyperchromic effect in the short wave-

(2) T. W. D. Gregory and W. H. Perkin, *J. Chem. Soc.*, **83**, 788 (1903).

(3) The infrared measurements were carried out by potassium bromide wafer technique using a Perkin-Elmer Infracord Model 137.

(4) Ultraviolet spectra were carried out using a Perkin-Elmer Spectracord Model 4000 in cyclohexane solutions.

(5) A. E. Gillam and E. S. Stern, *An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry*, London, Edward Arnold Ltd., 1955, p. 92.

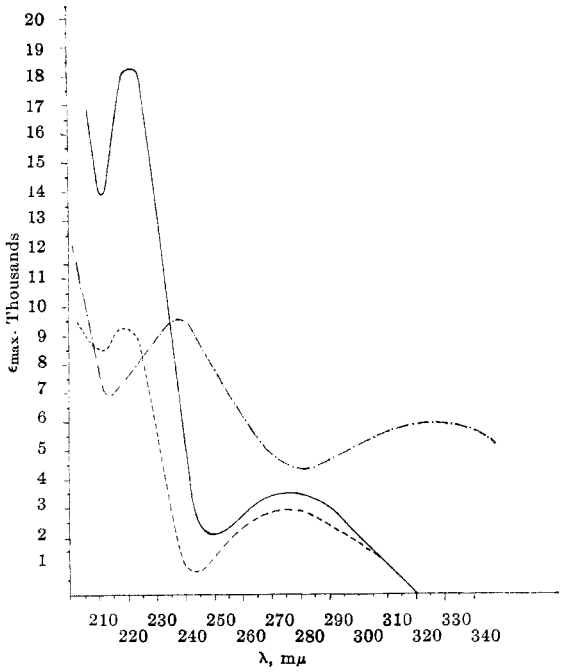


Figure 1

——— *N*-Phenylcitraconimide
—·—· *N*-Phenylitaconamide
-------- *N*-Phenylmaleimide

length band of the former imide is due to the methyl group,[5] which is a weak auxochrome. The ultraviolet spectrum of *N*-phenylitaconimide (Fig. 1) shows two bands at λ_{max} 238, 238 mμ, λ_{max} 9600, 6000, respectively, with a clear bathochromic shift.

The ultraviolet spectrum of the product, m.p. 125°, is very similar to that of *N*-phenylsuccinimide (*cf.* Fig. 2). This indicates that the product is most probably a cyclopropane[6] or a cyclohexane derivative. However, molecular weight determination and mixture melting point[2,7] prove that the product is the cyclopropane derivative.

The product, m.p. 212°, is a Δ^2-pyrazoline (III), as can be shown from the following facts: (*a*) elemental data; (b) the appearance of a band at 3333 cm.$^{-1}$ in its infrared spectrum, characteristic of N—H stretching frequency.[8] Δ^1-Pyrazolines are known to rearrange to Δ^2-pyrazolines when heated above their melting points.[9,10]

The fact that compounds IV and V are not interchangeable by heating under the above conditions, and also that *N*-phenylitaconimide was stable under the same conditions, indicates that most probably these two compounds (IV and V) are formed from II by the intermediate formation of a biradical (VI).

(6) Roger Adams, *Org. Reactions*, **8**, 385 (1954).
(7) W. H. Perkin, Jr., *J. Chem. Soc.*, **87**, 359 (1905).
(8) L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, London, Methuen and Co. Ltd., (1956) p. 215.
(9) Lee Irvin Smith and Kenneth L. Howard, *J. Am. Chem. Soc.*, **65**, 165 (1943).
(10) Lee Irvin Smith and W. B. Pings, *J. Org. Chem.*, **2**, 23 (1937).

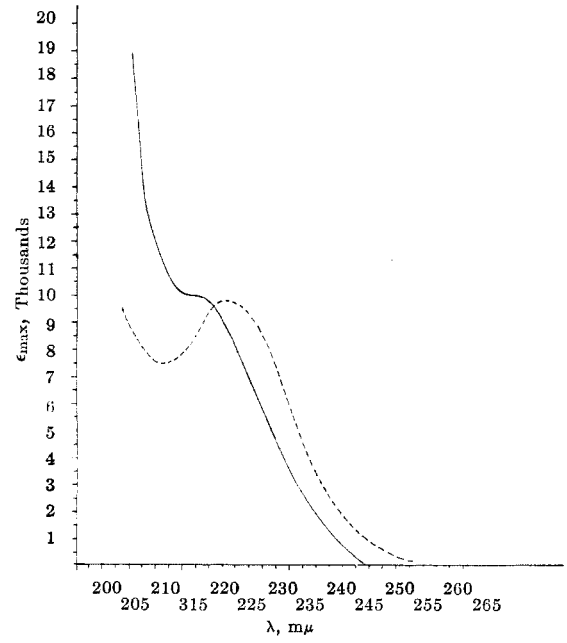
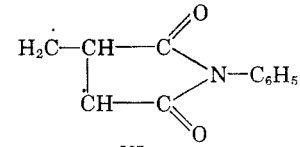


Figure 2

——— *N*-Phenylsuccinimide
-------- Cyclopropan-2,3,(*N*-phenyl)-dicarboximide



VI

However, III is obtained from II by a prototropic rearrangement.

EXPERIMENTAL[11]

Action of diazomethane on N-phenylmaleimide. The reaction was carried out as described by Mustafa *et al.*[1] and the melting point was the same (178°).

Thermal decomposition of Δ^1-pyrazoline-4,5(N-phenyl)-dicarboximide (II). The pyrazoline derivative (II) (0.5 g.) was heated in an oil bath at 180° under reduced pressure (0.2 mm.). Thermal decomposition took place with frothing and strong evolution of gases. The temperature was then allowed to stand between 160–170° for 6 hr., until most of the gases had evolved. The cold product was extracted several times with hot petroleum ether (b.p. 40–60°) filtered concentrated and cooled. The precipitated compound (0.16 g.) was repeatedly crystallized from petroleum ether (b.p. 40–60°) to give *N*-phenylcitraconimide in long pale yellow needles, m.p. 98° (yellow melt), undepressed on admixture with an authentic specimen.[12]

Anal. Calcd. for $C_{11}H_9NO_2$: C, 70.58; H, 4.85; N, 7.48. Found: C, 70.53; H, 4.92; N, 7.67.

The insoluble fraction was further extracted with petroleum ether (b.p. 70–80°), filtered, and concentrated. The colorless compound which crystallized on cooling was recrystallized from petroleum ether (b.p. 70–80°) to give the cyclopropane derivative V in long colorless needles (0.04 g.), m.p. 125°, undepressed on admixture with an authentic specimen.[2]

(11) The melting points are uncorrected. The analyses were carried out by Alfred Bernhardt, im Max-Planck-Institut, Mulheim (Ruhr), Germany.
(12) A. Reissert and F. Tiemann, *Ber.*, **19**, 623 (1886).

Anal. Calcd. for $C_{11}H_9NO_2$: C, 70.58; H, 4.85; N, 7.48, mol. wt., 187. Found: C, 70.73; H, 4.96; N, 7.43. mol. wt., (Rast's method) 175.6.

The residual solid which was insoluble in petroleum ether (b.p. 70–80°) was repeatedly crystallized from benzene to give Δ^2-pyrazoline (III) in colorless crystals (0.03 g.), m.p. 212° with slight evolution of gases (brown melt). When III was heated in a vacuum at 200°, it sublimed unchanged.

Anal. Calcd. for $C_{11}H_9N_3O_2$: C, 61.39; H, 4.19; N, 19.53. Found: C, 61.80; H, 4.55; N, 20.25.

When the above-mentioned thermal decomposition was carried out in a vacuum at 130° for 0.5 hr. no remarkable change was observed, and the starting material was recovered unchanged (melting point and mixture melting point).

When the decomposition was carried out under vacuum for 0.5 hr. at 180°, the following products were isolated from 0.5 g. of the starting pyrazoline derivative: (a) 0.14 g. of crystalline *N*-phenylitaconimide (m.p. 98°), (b) 0.03 g. of the Δ^2-pyrazoline (m.p. 212°), and (c) 0.5 g. of the unchanged starting material (m.p. 178°); the cyclopropane derivative (V) (m.p. 125°) could not be isolated.

Preparation of N-phenylitaconimide. A mixture of itaconic acid monoanilide[13] (20 g.), fused sodium acetate (2 g.), and excess of acetic anhydride (30 ml.) was heated at 80–90° for 1 hr. The reaction mixture was then poured portionwise with stirring into ice-cooled water. The precipitated solid product was filtered off, then washed with water, and crystallized first from benzene–petroleum ether (b.p. 40–60°) to give *N*-phenylitaconimide in yellow needles, m.p. 110° (lemon yellow melt).

Anal. Calcd. for $C_{11}H_9NO_2$: C, 70.58; H, 4.85; N, 7.48. Found: C, 70.61; H, 4.68; N, 7.68.

When this product was heated for 1 hr. just above its melting point, and the melt was triturated with hot petroleum ether (b.p. 40–60°), the mother liquor gave on concentration the starting material (melting point and mixture melting point).

Preparation of cyclopropane-2,3-(N-phenyl)dicarboximide. It was prepared according to Perkin *et al.*,[2] the melt in the last step was extracted with petroleum ether (b.p. 60–80°), and then the extract was concentrated and cooled, whereby colorless needles separated. By repeated crystallization from the same solvent, it was found that the melting point rose to 125°; Perkin *et al.*[2] gave a m.p. of 98°.

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(13) R. Anschütz and F. Reuter, *Ann.*, 254, 140 (1889).

Microbiological Transformation of Steroids. IX. The Transformation of Reichstein's Compound S by *Scenedesmus sp.* Diketopiperazine Metabolites from *Scenedesmus sp.*

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Species of algae generally hold the unique position found in higher plants of being able to synthesize their own protoplasm from carbon dioxide, sunlight and inorganic nitrate. Most physiological studies with algae have been concerned with this autotrophic method of growth.

We, on the other hand, were particularly interested in determining the metabolic capabilities of algae insofar as they could be used to transform steroids. Our search for conditions for rapid cell proliferation led us into a study of the area of heterotrophic nutrition. Several species were found which grew exceptionally rapidly in a heterotrophic medium containing carbohydrate and a complex organic nitrogen source. These particular algae grew equally well in shake flasks and in aerated and agitated laboratory fermentors of ten liters capacity. Since light enhanced growth in the heterotrophic medium, one might consider that these species of algae spanned both autotrophic and heterotrophic conditions and their nutrition could be classed as mixotrophic.

We have studied the action of *Scenedesmus sp.* J9A21, grown mixotrophically, on 4-pregnene-17α,-21-diol-3,20-dione (Reichstein's Compound S). To an actively growing culture in an NZ amine(Type A)-starch medium was added Compound S at a level of 100 mg./l. of medium. After forty-eight hour fermentation with illumination, aeration and agitation, the entire batch was extracted with chloroform.

The concentrated chloroform extract was chromatographed over Florisil and eluted with methylene chloride containing progressively increasing amounts of methanol. From the 100% methylene chloride fractions there was isolated a non-ultraviolet-absorbing, non-steroidal, crystalline mixture which contained nitrogen. Later fractions, eluted with 1%, 2% and 3% methanol contained mixtures of ultraviolet-absorbing products with migration rates like those of authentic Compound S, hydrocortisone, 4-pregnene-11α,17α,21-triol-3,20-dione, 4-pregnene-6β,17α,21-triol-3,20-dione and 4-pregnene-15β, 17α,21-triol-3,20-dione in the Shull system.[1] None of the steroid-containing fractions were crystalline.

The only steroidal product whose presence was established with certainty was 4-pregnene-6β,17α,-21-triol-3,20-dione. This was accomplished by degrading with sodium bismuthate[2] a sample of the combined steroidal fractions from a preliminary Florisil chromatographic purification. The resulting mixture was then separated on a toluene-propylene glycol-Chromosorb W partition column. A single crystalline product was isolated, whose melting point, rotation, ultraviolet spectrum and infrared spectrum were in good agreement with that of 4-androstene-6β-ol-3,17-dione. This was presumed to

(1) G. M. Shull, abstracts of papers, 126th Meeting of the American Chemical Society, Sept. 12–17, 1954, New York, p. 9A.

(2) C. J. W. Brooks and J. K. Norymberski, *Biochem. J.*, 55, 371 (1953).