amino acid in crude form. The product required two or three recrystallizations from water containing a trace of sulfur dioxide before it was pure.

2-Cyclohexanecarboxamido-3-(2,4-dimethoxy-3-methylphenyl)-propionic acid, 9.0 g. (0.026 mole), yielded 1.09 g. (20%) as microscopic colorless needles, m.p. 248° dec. (analytical sample, m.p. 254° dec.).

Anal. Calcd. for $C_{10}H_{13}NO_4$: C, 56.9; H, 6.2; N, 6.6. Found: C, 56.9; H, 6.4; N, 6.5.

2-Cyclohexanecarboxamido-3-(2,4-dimethoxy-5-methylphenyl)-propionic acid, 6.0 g. (0.017 mole), yielded 2.31 g. (64%) as colorless prisms, m.p. 256° dec. (analytical sample, m.p. 257° dec.).

Anal. Calcd. for $C_{10}H_{13}NO_4$: C, 56.9; H, 6.2; N, 6.6. Found: C, 57.2; H, 6.0; N, 6.9.

2-Cyclohexanecarboxamido-3-(2,4-dimethoxy-6-methylphenyl)-propionic acid, 9.0 g. (0.026 mole), yielded 1.60 g. (30%) as colorless prisms, m.p. 255–256° dec. (analytical sample, m.p. 260° dec.).

Anal. Calcd. for $C_{10}H_{13}NO_4$: C, 56.9; H, 6.2; N, 6.6. Found: C, 57.2; H, 6.3; N, 7.0.

The 46.0 g. of the crude mixture of 2-keto-3-benzamidocoumarin and 4-(2-acetoxybenzylidine)-2-phenyl-5-oxazolone, 6.0 g. of red phosphorus and 175 ml. each of hydriodic acid (sp. gr. 1.7) and glacial acetic acid were refluxed for 2 hours under an atmosphere of hydrogen. The mixture was filtered and evaporated under reduced pressure, hydrogen being drawn in through the capillary. The residue was suspended in 250 ml. of water, filtered and extracted with ether. The water phase was evaporated as above and the residual sirup dissolved in 150 ml. of water, the *p*H adjusted to approximately 7 by means of concentrated ammonium hydroxide solution and the suspension stored in the refrigerator. The product was collected, dissolved in 900 ml. of boiling water, decolorized and evaporated as above to about 500 ml. and refrigerated. The collected precipitate was washed on the filter with 25 ml. of water containing SO_2 and dried to produce 16.1 g. (36%) of *o*-tyrosine, m.p. 246° dec. as colorless crystalline material. The product was dissolved in 800 ml. of water and boiled on the hot plate until concentrated to 400 ml., treated with decolorizing carbon and refrigerated. The precipitate consisted of large crystals, m.p. 265° dec.[22] weighing 12.9 g. or 29% of the theoretical amount based on the quantity of salicylaldehyde used.

Paper Chromatography.—The R_f values of the amino acids were determined for the phenol–water system. Under the conditions which prevailed during the determination by the descending technique, L(−)tyrosine had an R_f value of 0.58; 2,4-dihydroxyphenylalanine, the 3-methyl-, 5-methyl-, 6-methyl-, 2,4-dihydroxyphenylalanines and *o*-tyrosine had R_f values of 0.37,[2] 0.50, 0.50, 0.44 and 0.71, respectively.

Enzyme Activity Determinations.—The enzyme studies were done essentially like those reported on a previous occasion.[2] 2,4-Dihydroxy-3-methylphenylalanine was used at levels of 0.1 to 2.0 mg. and when compared to tubes containing one-half these levels of L(−)tyrosine it was found that the 3-methylamino acid was oxidized at a somewhat greater rate than tyrosine, but to a brick-red color. The same levels of 2,4-dihydroxy-5-methylphenylalanine were used and all tubes gave the same reading after 6 hours as were given by the enzyme blanks. When tyrosine was also added the color production was equivalent to the tyrosine added. Graded levels of 2,4-dihydroxy-6-methylphenylalanine (0.1 to 1.5 mg.) progressively inhibited the oxidation of 0.25 mg. of L(−)tyrosine. The inhibition caused by 0.5 mg. of 6-methylamino acid was progressively relieved by the addition of graded amounts (0.15 to 1.0 mg.) of tyrosine.

(22) Previous reports of the m.p. were between 249 and 251° dec.

ROCHESTER, NEW YORK

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Investigation of the Chemical Nature of Gonyleptidine

BY LOUIS F. FIESER AND MARIA ISABEL ARDAO

RECEIVED JULY 18, 1955

The major component of the antibiotic gonyleptidine, a yellow pigment from the secretion of a South American arachnid, was identified by isolation of the hydroquinone diacetate as 2.3-dimethyl-1,4-quinone. Because of correspondence of infrared absorption bands with those of gonyleptidine, and because of even higher bacteriostatic potency, 2,5-dimethyl-1,4-quinone and 2,3,5-trimethyl-1.4-quinone seemed likely minor companions, and various methods of fractionation were tested on known mixtures of the three synthetic quinones. That finally applied to 115 mg. of gonyleptidine involved. in the first step, reaction with 2,3-dimethylbutadiene for selective conversion of 2.3-dimethyl-1,4-quinone to an adduct, which remained in the neutral fraction when the unreacted quinones were reduced with hydrosulfite and extracted with alkali. After reoxidation, the quinone mixture was submitted to Thiele acetoxylation. The 2.5-dimethyl-1,4-quinone present yielded the non-steam-volatile 2,5-dimethyl-1,3,4-triacetoxybenzene, and 2,3,5-trimethyl-1.4-quinone was isolated from the steam distillate. Thioacetic and β-thiopropionic acid derivatives of the alkyl-1.4-quinone were prepared incidentally and some of them have been found to be bacteriostatic. New observations concerning the scope of the Thiele reaction are presented.

Clemente Estable and associates of the Instituto de Investigación de Ciencias Biológicas, Ministerio de Salud Pública, Montevideo, Uruguay, discovered that a yellow aqueous fluid secreted by the South American arachnid *Gonyleptide* has remarkable antibiotic properties.[1] The yellow pigment, named gonyleptidine, is bacteriostatic, *in vitro*, against Gram positive and Gram negative bacteria and protozoa. The initial discovery was made in consequence of striking biological actions exerted by minute amounts of material reaching microörganisms at one site in the laboratory by air transport from arachnids at a distant site.

Withdrawal of secretion from an arachnid by capillary pipet affords only about 0.01 ml. of a yellow aqueous suspension containing 2–3 mg. of pigment per ml., and after one or two repetitions of the process the arachnid fails to yield significant further amounts of fluid. Thus the amount of antibiotic pigment that can be collected in a given season from thousands of arachnids is small. The research thus reached a point where identification of the active principle or principles of gonyleptidine become imperative. Exploratory investigations conducted by one of us at Montevideo established that on distillation of extract from the frozen state gonyleptidine distils first as a bright yellow crystalline substance of melting point about 12° and is followed by a water solution of the pigment. The substance shows selective ultraviolet absorption at 255 mμ with the extinction coefficient $E^{1\%}$ 1400 (water). Antibiotic activity against various micro-

(1) C. Estable, M. I. Ardao, N. P. Brasil and L. F. Fieser, THIS JOURNAL, **77**, 4942 (1955).

organisms is observed at concentrations ranging from 1–2 γ/ml. to 50–100 γ/ml. An organic chemical investigation of gonyleptidine being beyond the scope of activities of the institute at Montevideo, support was secured from the Rockefeller Foundation to enable a member of the staff (M. I. A.) to conduct such research in this Laboratory.

The first observation of the present research was that treatment of yellow gonyleptidine extract, after distillation from the frozen state, with sodium hydrosulfite causes separation of a white solid, evidently a hydroquinone, of m.p. about 185°. For further purification this was converted to the corresponding diacetate; chromatography of the diacetate showed the material to be inhomogeneous, but the total supply available was sufficient only for isolation of the major component of the mixture, a substance melting at 105–106°. Combustion analysis, acetyl determination, and determination of molecular weight established that the substance has the formula $C_{12}H_{14}O_4$ and is the hydroquinone diacetate of a quinone of the formula $C_8H_8O_2$. The yellow color of gonyleptidine indicates that the major component is a para quinone; hence it must be one of the following substances: 2-ethyl-1,4-quinone, 2,3-, 2,5-, or 2,6-dimethyl-1,4-quinone. The previously undescribed 2,3-dimethylhydroquinone diacetate was found to be identical with the diacetate isolated. The major quinone of gonyleptidine is thus identified as 2,3-dimethyl-1,4-quinone (I). Synthetic I was subsequently found in Montevideo to have antibiotic properties comparable with gonyleptidine.

The isolation of a derivative had involved a reduction in the first step, and hence the experiment did not establish whether or not the component identified was initially present entirely in the quinone form, although the yellow color, ultraviolet absorption and volatility of gonyleptidine are properties attributable only to the quinone. However, evidence was obtained in a polarographic study kindly undertaken by Professor J. J. Lingane and Dr. Imanuel Bergman. 2,3-Dimethyl-1,4-quinone was compared with gonyleptidine in aqueous solution at *p*H 5.2 and *p*H 6.9; the solutions had to be prepared in the absence of air because otherwise decomposition to a white insoluble product occurred. Both substances gave the same logarithmic curve when titrated reductively as they did when titrated oxidatively, which shows that the species initially present was solely the quinone form. The curves had the characteristic two-electron slope of 0.0295, indicative of the absence of a semiquinone. The normal oxidation–reduction potential found for 2,3-dimethyl-1,4-quinone was $E_0^{25°} = 0.588 \pm 2$ v., a value close to that reported for the isomeric 2,5-dimethyl-1,4-quinone, $E_0^{25°} = 0.590$ v.[2] The polarogram of gonyleptidine showed that the major component quinone or quinones also has or have the potential 0.588–0.590 v. characteristic of the dimethyl-1,4-quinones but that another quinone of somewhat lower potential is present to the extent of 10–15%. Since each substituent methyl group lowers the potential of *p*-benzoquinone by about 55 mv., the estimated potentials of 2,3,5-trimethyl-1,4-quinone and of duroquinone are 0.534 and 0.479 v., respectively; hence the other component of the natural pigment could be one of these substances. Duroquinone appeared unlikely as a component because of its relatively high melting point (111°) and lack of water solubility; the low-melting, water-soluble trimethylquinone (m.p. 29°) seemed a reasonable possibility.

A further clue to the identity of the companion quinones was obtained by analysis of the infrared absorption spectrum of gonyleptidine in comparison with the spectra of *p*-benzoquinone, its methyl derivatives and 2-ethyl-1,4-quinone. The pertinent data are summarized in the spectrum and caption of Fig. 1. Three bands (c, e, i) in the spectrum of the pigment are characteristic of 2,3-dimethyl-1,4-quinone alone, and bands b and h are characteristic exclusively of the 2,5-isomer and of trimethylquinone, respectively. Summarizing the average results of preliminary studies with representative microörganisms, Dr. Estable reported 2,5-dimethyl-1,4-quinone to be twice as active as gonyleptidine against Gram positive bacteria and four times as active against Gram negative bacteria. The trimethylquinone is only slightly less potent and is more active than 2,3-dimethyl-1,4-quinone.

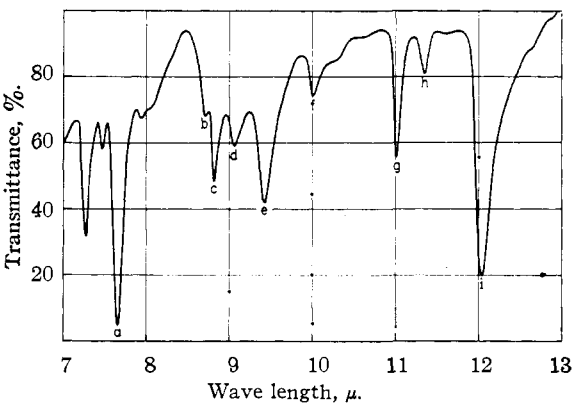


Fig. 1.—Infrared spectrum of gonyleptidine (Baird spectrophotometer). Bands characteristic of 2,3-dimethyl-1,4-quinone: *a* (also characteristic of *p*-benzoquinone), *c*, *d* (also 2,3,5-trimethyl-1,4-quinone), *e*, *i*; of 2,5-dimethyl-1,4-quinone: *b*, *f* (also *p*-toluquinone), *g* (also shown by the 2,6-isomer, with which, however, the band corresponding to *a* is double-peaked): of 2,3,5-trimethyl-1,4-quinone; *d* (also the 2,3-dimethylquinone), *h*. Ethylquinone has a strong band at 11.0 μ, but bands in the region 7–8 and 9–10 μ are considerably displaced from the positions shown in the figure.

The evidence from both spectrographic analysis and biological activity so strongly suggested that the minor components of gonyleptidine are 2,5-dimethyl-1,4-quinone and 2,3,5-trimethyl-1,4-quinone that we undertook development of a procedure for separation of the specific mixture indicated. Our supply of insect extract amounted to only 43 ml. and could be expected to contain only a few milligrams of the minor components; the season when fresh arachnids could be collected was several months away. Hence the procedure of separation that we sought would have to involve efficient, high-

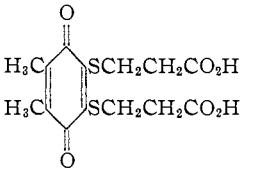
(2) J. B. Conant and L. F. Fieser, THIS JOURNAL, **45**, 2194 (1923).

yield techniques or reactions. Chromatography was tried briefly but appeared inadequate. Countercurrent distribution between petroleum ether and aqueous buffers in a 32-plate Craig machine was tried with unpromising results. Determination of concentration in the two phases by ultraviolet spectrography indicated rapid disappearance of quinone pigment, probably owing to decomposition of 2,3-dimethyl-1,4-quinone.

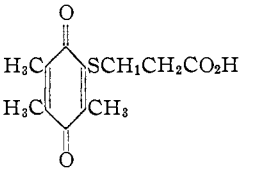
We became fully aware of the high sensitivity of 2,3-dimethyl-1,4-quinone only in the course of preparing a large batch of material for pharmacological processing in Montevideo. Emerson and Smith[3] prepared the quinone from 2,3-dimethylaniline sulfate in 33% yield by dichromate oxidation, extraction with ether, and steam distillation at diminished pressure. They state that distillation at 100 mm. pressure was necessary because the quinone is thermolabile. We found this process tedious and uncertain, and investigation of the properties of 2,3-dimethyl-1,4-quinone showed that the substance is not labile to heat alone but very sensitive to mineral acids and to light. A bright yellow aqueous solution acquires a dull, dirty appearance when acidified with hydrochloric acid, and discoloration also occurs at room temperature at *p*H 5–7. However the stability is satisfactory at *p*H 3.9, and if a solution is buffered with potassium acid phthalate steam distillation can be conducted satisfactorily at atmospheric pressure. Gonyleptide extract has a *p*H of approximately 4. A reliable preparative method was found in dropwise addition of a solution of 2,3-dimethyl-4-aminophenol hydrochloride to a boiling solution of ferric chloride and hydrochloric acid; the quinone steam distils as it is formed and is thus protected from the destructive action of acid. Yields up to 80% were realized.

2,3-Dimethyl-1,4-quinone appears to be much more sensitive to light than thymoquinone,[4] and somewhat more sensitive than 4-methyl-1,2-quinone,[5] both of which are converted to dimers. Conversion of 2,3-dimethyl-1,4-quinone to a white, ether-insoluble polymer is observable in a few minutes on exposure of a yellow layer of solid to bright sunlight.

With recognition of the lability of the major component of gonyleptidine, we sought a method of processing that in the first step would provide chemical stabilization. One idea was to treat the mixture with thioglycolic or β-thiopropionic acid and then oxidize the resulting substituted hydroquinone to the quinone; from prior work in both the benzo-[6] and naphthoquinone[7] series it could be inferred that all free positions in the quinone nucleus would be substituted. β-Thiopropionic acid was selected in preference to thioglycolic acid both to avoid lactonization of the hydroquinone and because of the greater increase in molecular weight. This reagent does indeed add as expected to all three dimethylquinones and to trimethylquinone, and the addition products are oxidizable to quinones. The labile 2,3-dimethyl-1,4-quinone yields a stable, red, high-melting (172°) tetrasubstituted derivative II; low melting, volatile 2,3,5-trimethyl-



II



III

1,4-quinone (m.p. 29°) yields a non-volatile acid derivative III melting at 104°. The monoacid III should be easily separable from the diacid II and the 2,5-isomer by countercurrent buffer distribution, but separation of a small amount of diacid derivative of 2,5-dimethyl-1,4-quinone from a large amount of II could be expected to present difficulties. Furthermore, the yields in the process of sulfhydryl addition and oxidation were poor. We therefore deferred actual trial of the method. However, samples of 2,3,5-trimethylquinone-6-β-thiopropionic acid (III) and the corresponding thioacetic acid derivative were found in Montevideo to have interesting bacteriostatic properties, particularly the thiopropionic acid derivative, and several other of these water-soluble, stable, unreactive substances were synthesized for biological investigation.

We next explored the Thiele reaction,[8,9] in an approach that was necessarily empirical since the limits in the acid-catalyzed addition of acetic anhydride to quinones have not been fully defined. Using sulfuric acid as catalyst, Thiele[8] obtained pure hydroquinone triacetate from *p*-benzoquinone in 80% yield. In the case of α-naphthoquinone, boron fluoride etherate gives a better result (81% yield) than sulfuric acid (74.5%),[10] but the Lewis acid is less satisfactory than sulfuric acid for the reaction of *p*-benzoquinone. Erdtman[11] noted that 2,6-dimethyl-1,4-quinone undergoes sulfuric acid-catalyzed Thiele reaction but that 2,6-dimethoxy- and 2,5-dimethoxy-1,4-quinone do not react. From this evidence the only safe prediction possible concerning the suspected components of gonyleptidine is that 2,3-dimethyl-1,4-quinone should react, as indeed it does. In contrast to 2,5-dimethoxy-1,4-quinone, 2,5-dimethyl-1,4-quinone undergoes smooth Thiele reaction (BF_3-catalyzed) and affords a nicely crystalline triacetate V of double the molecular weight of the quinone and in 91% yield; this is thus a useful characterizing derivative. It is converted by hydrolysis and oxidation into a hydroxyquinone VI of a type that we thought might be of use in separations.

Thus in alkaline buffer the anion absorbs at 530 mμ (E_{mol} 1970) and is distinguishable from that of the isomeric 5-hydroxy-2,3-dimethyl-1,4-quinone, which absorbs at 490 mμ (E_{mol} 1590). Incidentally,

(3) O. H. Emerson and L. I. Smith, THIS JOURNAL, **62**, 141 (1940).

(4) C. Liebermann, *Ber.*, **10**, 2177 (1877); C. Liebermann and M. Ilinski, *ibid.*, **18**, 3193 (1885); K. Lagodzinski and M. Mateescu, *ibid.*, **27**, 958 (1894).

(5) R. Willstätter and F. Müller, *Ber.*, **44**, 2171 (1911).

(6) J. M. Snell and A. Weissberger, THIS JOURNAL, **61**, 450 (1939); A. Blackhall and R. H. Thompson, *J. Chem. Soc.*, 1138 (1953).

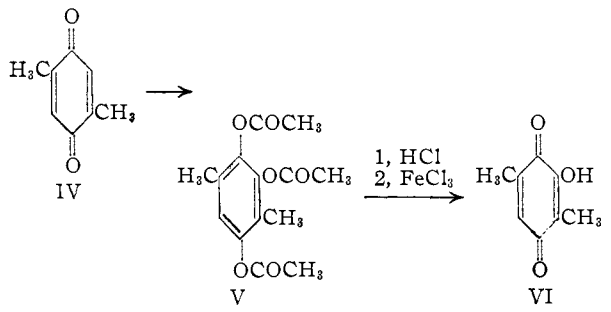
(7) L. F. Fieser and R. B. Turner, THIS JOURNAL, **69**, 2335 (1947).

(8) J. Thiele, *Ber.*, **31**, 1248 (1898).

(9) J. Thiele and E. Winter, *Ann.*, **311**, 341 (1900).

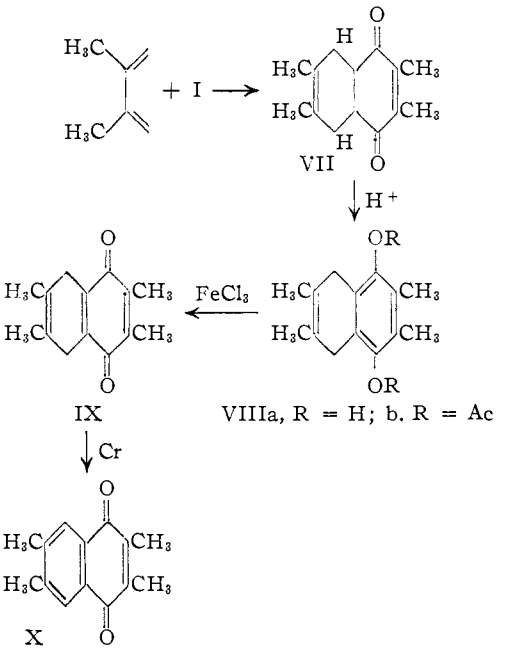
(10) L. F. Fieser, THIS JOURNAL, **70**, 3165 (1948).

(11) H. Erdtman, *Svensk. Kem. Tids.*, **44**, 135 (1932) (*C. A.*, **26**, 4803 (1932)).



the logarithmic extraction constants, pE,[12] found for 5-hydroxy-2,3-dimethyl-1,4-quinone (pE = 3.70) and for 2-hydroxy-1,4-naphthoquinone (3.17) are both lower than the values calculated from the empirical equation for *n*- and *i*-alkylhydroxynaphthoquinones. The isomeric hydroxydialkylquinones should be separable by esterification and extraction. The quinone VI, with a methyl substituent adjacent to the hydroxyl group, is resistant to Fischer esterification in overnight reaction with methanol–boron fluoride etherate, whereas under the same conditions hydroxybenzoquinones with no substituent adjacent to the hydroxyl group are readily esterified. However, hydroxybenzoquinones are generally so sensitive and easily decomposed that a separation involving them would be subject to considerable uncertainty.

Whereas, as already noted, 2,6-dimethyl-1,4-quinone undergoes Thiele reaction in the presence of sulfuric acid, it was recovered unchanged after attempted acetoxylation in the presence of boron fluoride etherate. The observation shows both that boron fluoride is a less potent catalyst than sulfuric acid and that 2,6-dimethyl-1,4-quinone is less reactive than the 2,5-isomer. In trials of sulfuric acid-catalyzed Thiele reaction on 2,6-dichloro-1,4-quinone and 2,3,5-trimethyl-1,4-quinone, starting material was recovered unchanged. That 2,5-dimethyl-1,4-quinone undergoes the Thiele reaction in high yield whereas 2,3,5-trimethyl-1,4-quinone can be recovered quantitatively from a comparable reaction mixture affords an efficient means of separating these suspected gonyleptidine components. If the reaction mixture is poured into water and the solution distilled, the unreacted trimethylquinone passes quantitatively into the distillate and the water-soluble, non-steam volatile dimethyltriacetoxy compound V can be recovered from the colorless solution in the boiling flask. A mixture of 2,5-dimethyl-, 2,6-dimethyl- and 2,3,5-trimethylquinones should be separable in a series of two Thiele reactions, the first in the presence of boron fluoride, the second utilizing sulfuric acid. For present purposes it seemed desirable to remove the 2,3-dimethyl-1,4-quinone prior to the Thiele reaction by a selective, stabilizing reaction. The Diels–Alder reaction seemed ideal for the purpose, since dienes add at room temperature to quinones having one unsubstituted enedione system, but when the system carries a blocking methyl group addition occurs only under forcing conditions.[13] Under conditions similar to those worked out for preparation of the *p*-benzoquinone–butadiene adduct (in acetic acid at room temperature)[10] 2,3-dimethyl-1,4-quinone and 2,3-dimethylbutadiene form the crystalline adduct VII in nearly quantitative yield. This adduct can be characterized as such, or as the hydroquinone VIIIa to which it is isomerized by acid, its diacetate VIIIb, the quinone IX, or the more stable naphthoquinone X (m.p. 167°). The molecular weight of VIIIb is 2.2 times that of the starting material I.



The procedure of separation was applied to a solution of 100 mg. of 2,3-dimethylquinone and 25 mg. each of 2,5-dimethyl- and 2,3,5-trimethylquinone in 150 ml. of water. This was saturated with salt, and the yellow pigment extracted with ether and let react for 20 hr. with 2,3-dimethylbutadiene. The material was collected in ether and the solution washed until neutral and then shaken with hydrosulfite; this discharges the color but does not affect the adduct VII. The 2,5-dimethyl- and 2,3,5-trimethylhydroquinones produced were extracted with alkali and the ethereal solution of the neutral fraction processed for recovery of the adduct VII, the yield of which was 150 mg. (94%). The alkaline liquor had been drained into a funnel containing ether and mineral acid; ferric chloride was added and the quinone pigment was extracted, submitted to the Thiele reaction, and the mixture steam distilled. 2,3,5-Trimethylquinone was isolated as the high melting hydroquinone in 78% yield and the yield of the triacetate V was 64%. The procedure, which is not only qualitative but roughly quantitative, was then applied to 43 ml. of distilled gonyleptide extract, which afforded 115 mg. of yellow pigment. 2,3-Dimethylquinone adduct VII of the same purity as in the trials with known mixtures was identified by melting point comparison both as such and as the naphthoquinone XVIII. The 2,5-dimethyltriacetoxy derivative from the 2,5-component was isolated and identified by mixed melting point and infrared comparison, and 2,3,5-

(12) L. F. Fieser, M. G. Ettlinger and G. Fawaz, THIS JOURNAL, **70**, 3228 (1948).

(13) L. F. Fieser and A. M. Seligman, *ibid.*, **56**, 2690 (1934).

trimethylquinone was determined from the extinction coefficient at 260 mμ and identified by complete correspondence of the infrared spectrum and from the mixed melting point of the hydroquinone. On the assumption that the losses were the same as in the best model experiment, the yields obtained account for the following quantities of components in the 115 mg. of pigment processed: 71 mg. of 2,3-dimethyl-, 11 mg. of 2,5-dimethyl-, and 15 mg. of 2,3,5-trimethylquinone. Not more than a trace of non-quinone component could have been present.

If this isolation procedure were applied to a mixture containing both a 2,3-disubstituted and a 2-substituted quinone,[14] the mixture of diene adducts could be converted (by high-yield steps) to the mixture of naphthoquinones, only one of which could react with thioacetic or thiopropionic acid to give a bicarbonate-extractable product.

Acknowledgment.—We heartily acknowledge our indebtedness to Professor Estable and his staff at Montevideo for enabling us to investigate the chemistry of the antibiotic principle discovered in Uruguay and for their coöperation in the course of the work. For grants permitting or facilitating participation of both of us in the experimentation, we are indebted to the Rockefeller Foundation (M. L. A.) and to Research Corporation (L. F. F.). Grants from the National Institutes of Health and the National Science Foundation provided part-time assistants in preparation of large lots of materials for biological testing.

Experimental

Isolation of **2,3-Dimethylhydroquinone Diacetate.**—Approximately 38 ml. of gonyleptide extract was treated with 1 g. of sodium hydrosulfite, when the yellow color was at once discharged, and extracted with peroxide-free ether. The ethereal extract was dried and evaporated until most of the ether was removed, and petroleum ether was added. White needles of a hydroquinone separated, m.p. about 185°, yield 80 mg. Acetylation of 67 mg. of this material with acetic anhydride and pyridine afforded 81 mg. of hydroquinone diacetate, which Dr. Wei-Yuan Huang purified by chromatography and crystallization. The main component isolated melted at 105–106° and did not depress the m.p. of synthetic 2,3-dimethylhydroquinone diacetate (105–106°); the infrared spectra also showed exact correspondence.

Anal. Calcd. for $C_{12}H_{14}O_4$ (222.23): C, 64.85; H, 6.35; acetyl, 37.83. Found: C, 64.69; H, 6.46; acetyl, 38.02, mol. wt., 237.

2,3-Dimethyl-1,4-quinone (I).—Two 76.5-g. batches of 2,3-dimethylphenol were coupled with diazotized sulfanilic acid and the dye reduced with hydrosulfite by a standardized procedure.[15] The two batches of 2,3-dimethyl-4-aminophenol were collected together and the combined product dissolved in a solution of 265 ml. of concd. hydrochloric acid and 2 g. of stannous chloride dihydrate in 500 ml. of water. The solution was filtered through a pad of Norit and diluted to a volume of 1250 ml. If the yield were quantitative, 100 ml. of this solution would contain 0.1 mole of the aminophenol hydrochloride.

For oxidation, conducted in a subdued light, 100 ml. of hot aminophenol hydrochloride solution was added during 15 minutes to a boiling solution of 65 g. of ferric chloride hexahydrate. The quinone formed steam distils at the rate the solution is added. When no more quinone distilled, the condenser was rinsed into the receiver with ether, and the distillate was saturated with salt (0.2 g. per ml.) and extracted with ether. The aqueous layer was separated and discarded and the yellow ethereal solution filtered through a cone of anhydrous sodium sulfate. The dried solution was evaporated on the steam-bath with avoidance of overheating, and the last traces of ether were removed by evacuation at the water pump after cooling. The residual yellow oil solidified easily and was substantially pure 2,3-dimethyl-1,4-quinone; yield 9.6 g. (71% over-all). The yield from the pure aminophenol hydrochloride was 80%. The substance is readily soluble in petroleum ether, but crystallization from this solvent (b.p. 30–60°) at 5° gives successive crops of clear yellow product, m.p. 55°. Residual mother liquors that had acquired a reddish color were evaporated and the product dissolved in ether. The ethereal solution was run dropwise from a separatory funnel into a distilling flask containing a briskly boiling 5% aqueous solution of potassium acid phthalate; saturation of the distillate with salt and separation of the ether layer afforded pure, clear yellow 2,3-dimethyl-1,4-quinone.

The bright yellow aqueous solution of this quinone turns purple soon after addition of bicarbonate solution, and it is also unstable to sodium acetate or to a phosphate buffer of *p*H 7. When the yellow solution is heated under reflux, yellow droplets of oil appear in the condenser, but if the solution is treated with a trace of hydrochloric acid the solution soon acquires a dull brown color, the appearance of yellow oil droplets in the condenser ceases, and eventually dark products of decomposition separate.

The quinone (200 mg.) was dissolved in a few ml. of ether and the solution was distributed over the walls of a 100-ml. flask, allowed to evaporate and the flask inverted and exposed to bright sunlight. Within a few minutes blanching of the yellow pigment was observable in some areas, and when the ether was poured down the walls a film of white solid remained undissolved. By repetition of the process the quinone was practically all converted into white polymer in the course of one hour.

2,3-Dimethylhydroquinone diacetate crystallized from methanol–water in long needles, m.p. 105–106°.

Anal. Calcd. for $C_{12}H_{14}O_4$ (222.23): C, 64.85; H, 6.35. Found: C, 65.10; H, 6.58.

2,3,5-Trimethyl-1,4-quinone.—A suspension of 152 g. of trimethylhydroquinone[16] (m.p. 169–170°) in 1 l. of acetic acid was stirred mechanically in a 2-l. flask, heated until the solid had dissolved, and chilled in an ice-bath until the temperature had dropped to 40°. A cooled solution of 108 g. of sodium dichromate dihydrate in 250 ml. of acetic acid was then run in in the course of about 25 min. with constant ice cooling to control the temperature to 35–40°. After stirring for 5 min. more the solution was diluted with water and cooled. After suitable further dilution the mixture was extracted with ether three times and the bright yellow extract was washed free of acid with salt solution, filtered through sodium sulfate and the solvent evaporated. The residual pure yellow oil solidified readily and melted at 29–30°, in agreement with the constant for pure material reported by Smith.[17] The m.p. was not changed on crystallization from petroleum ether. The yield was 128.3 g. (86%).

2,3-Dimethyl-1,4-quinone-5,6-di-β-thiopropionic Acid (II).—To a stirred suspension of 1.4 g. of 2,3-dimethyl-1,4-quinone in 50 ml. of water 2.1 g. of β-mercaptopropionic acid was added. The solid soon dissolved, the color faded, and white hydroquinone soon began to separate and in one hour formed a heavy paste containing only a few specks of dark material. The solid was collected, washed into a beaker with water, and 1.5 g. of sodium bicarbonate was added. On stirring and heating, the solid nearly all dissolved and the colorless solution was filtered from a trace of insoluble material, acidified, and the white precipitate collected after cooling. The moist solid was dissolved in 10 ml. of hot acetic acid and the solution was let cool slightly and treated with 10 ml. of a solution 1 *N* in both ferric chloride and hydrochloric acid. When the red solution was cooled, diluted with 50 ml. of water, the quinone was obtained as a

(14) Flour beetle extract has been shown to contain ethylquinone (80–90%), *p*-toluquinone and methoxyquinone: P. Alexander and D. H. R. Barton, *Biochem. J.*, **37**, 463 (1943); R. H. Hackman, M. G. M. Pryor and A. R. Todd, *ibid.*, **43**, 474 (1948); J. D. Loconti and L. M. Roth, *Ann. Ent. Soc. Am.*, **46**, 281 (1953).

(15) L. F. Fieser and M. Fieser, THIS JOURNAL, **57**, 491 (1935); L. F. Fieser, "Organic Syntheses," Coll. Vol. **2**, John Wiley and Sons, New York, N. Y., 1943, p. 35.

(16) We are indebted to Merck and Co., Inc., and Hoffmann La Roche, Inc., for generous supplies of this material.

(17) L. I. Smith, THIS JOURNAL, **56**, 472 (1934).

crystalline red solid, m.p. 167°; yield 0.96 g. (28%). The substance is very soluble in cold methanol, slightly soluble in ether, very sparingly soluble in benzene or chloroform. The material crystallized from water (about 200 ml.) in small, deep red needles, m.p. 171–172°, unchanged on recrystallization.

Anal. Calcd. for $C_{14}H_{16}O_6S_2$ (344.40): C, 48.84; H, 4.67. Found: C, 48.85; H, 4.80.

When the thiol addition was done in aqueous ethanol the quinone could not be obtained crystalline.

2,5-Dimethyl-1,4-quinone-3,6-di-β-thiopropionic Acid.—A solution of 1.4 g. of the quinone in 15 ml. of hot 95% ethanol was cooled to produce a suspension and a solution of 2.1 g. of β-mercaptopropionic acid was added. The quinone soon dissolved to a brown solution which, after 1 hr. when it had become nearly colorless, was treated with 20 ml. of ethanol and 40 ml. of 1 *N* ferric chloride–hydrochloric acid. The solution was diluted, extracted with ether, and the extract washed several times with saturated salt solution, dried and evaporated to a small volume. Cooling and scratching gave crystalline material, m.p. 145–150°; yield 0.67 g. (20%). The quinone crystallized nicely from methanol–water in shiny orange plates, m.p. 154–156°, recrystallized, 155–157°.

Anal. Calcd. for $C_{14}H_{16}O_6S_2$ (344.40): C, 48.84; H, 4.67. Found: C, 49.25; H, 4.86.

2,6-Dimethyl-1,4-quinone-3,5-di-β-thiopropionic acid was obtained by the aqueous procedure described for II in yield of 0.82 g. (24%), m.p. 127–129°. It was crystallized (twice) by diluting a solution in ether with petroleum ether and evaporating to a small volume; well-formed, orange needles separated, m.p. 131–132°.

Anal. Calcd. for $C_{14}H_{16}O_6S_2$ (344.40): C, 48.84; H, 4.67. Found: C, 48.96; H, 4.67.

The yield was somewhat less when ethanol–water was used as solvent in thiol addition, and much poorer when ethanol alone was used.

2,3,5-Trimethyl-1,4-quinone-6β-thiopropionic acid (III) was prepared from 8.8 g. of the quinone by the aqueous procedure (see II). The bicarbonate solution of the hydroquinone required a little hydrosulfite for decoloration. The precipitated hydroquinone was collected and dried (yield 81%), although this was of no advantage. Oxidation in hot acetic acid with ferric chloride gave crystalline, directly pure product, m.p. 103–104°, in over-all yield of 76%. The quinone crystallized in round clusters of orange-yellow prisms after an ethereal solution had been diluted with ligroin and concentrated, m.p. 103–104°.

Anal. Calcd. for $C_{12}H_{14}O_4S$ (254.23): C, 56.69; H, 5.55. Found: C, 56.35, 56.34; H, 5.69, 5.74.

2,3,5-Trimethyl-1,4-quinone-6β-thioacetic Acid.—The addition reaction was conducted as described by Snell and Weissburger[6]: a solution of 1.5 g. of the quinone in 15 ml. of 95% ethanol was treated with 0.9 g. of thioglycolic acid in 10 ml. of water and let stand overnight. The dark solution was treated with 22 ml. of 1 *N* ferric chloride–hydrochloric acid, diluted with 20 ml. of water, and extracted with ether. The extract was washed with salt solution and then extracted with 5% sodium bicarbonate solution. The extract was acidified and the quinone extracted with ether; evaporation of the dried solution afforded 1.7 g. of orange solid. The product is readily soluble in acetone, fairly soluble in benzene, and sparingly soluble in ligroin. For crystallization it was dissolved in ether, ligroin (60–90°) was added, and the solution was boiled down to the point of saturation; clusters of orange, prismatic needles separated, m.p. 130–131° (given[6] 126–127°); yield 1.3 g. (57%).

The neutral fraction (0.5 g.) recovered from the ether solution after bicarbonate extraction and two crystallizations from benzene–ligroin (Norit) afforded colorless needles, m.p. 196–197°, of the **hydroquinone lactone**.

Anal. Calcd. for $C_{11}H_{12}O_3S$ (224.21): C, 58.52; H, 5.40. Found: C, 58.79; H, 5.46.

2-Methyl-1,4-quinone-tri-β-thiopropionic Acid.—A mixture of 6 g. of *p*-toluquinone, 22 g. of β-thiopropionic acid and 50 ml. of water was stirred until the quinone had all dissolved and the initial dark color had largely bleached and the solution was let stand overnight, when it had become colorless and a little oil had separated. Salt was added to saturation and the mixture extracted twice with ether. The ethereal solution was extracted exhaustively with bicarbonate; the neutral fraction when dried and evaporated yielded 1 g. of toluhydroquinone, which formed clusters of white needles from benzene; m.p. 121–122°. Acidification of the bicarbonate extract gave an oil, which largely dissolved at steam-bath temperature. The hot solution was acidified and cooled and the red oil that separated extracted with ether. Evaporation gave a red semi-solid which when boiled with water lost the red color and changed to a suspension of brownish solid; evidently thioglycolic acid present had reduced the quinone to the hydroquinone. The solid was collected (12.6 g., m.p. 180–182°) and a part of it was dissolved in methanol and reoxidized. Extraction with ether again gave a red oil, which resisted initial attempts with ether, water and other solvents to cause it to solidify. Eventually it was found that if a hot aqueous solution is let cool and then let stand for several hours the oil that initially separated changed to a solid. Once solid was obtained, the quinone crystallized well in the normal fashion and it proved to be a high-melting substance of moderate solubility in hot water. It formed a crust of shiny, dark red prisms melting constantly at 165–166°. Material retained in the mother liquor can be recovered by addition of salt.

Anal. Calcd. for $C_{16}H_{18}O_8S_3$ (434.50): C, 44.23; H, 4.18. Found: C, 44.34; H, 4.32.

1,4-Quinone-2,3,5,6-tetra-β-thiopropionic acid (m.p. 194°), was prepared essentially as described by Blackhall and Thompson[6] except that quinone was treated with 4 equivalents of β-thiopropionic acid in water and the filtered, colorless solution oxidized with ferric chloride.

2,3-Dimethyl-1,4,5-triacetoxybenzene.—A mixture of 1 g. of 2,3-dimethyl-1,4-quinone, 10 ml. of acetic anhydride and 1 ml. of boron fluoride etherate was let stand at 26° for 2 hr., water was added, and after the excess anhydride had been hydrolyzed the product was extracted with ether, the solution was washed neutral, dried and evaporated. The residual solid (1.8 g., 88%) was dissolved in ether and, after largely displacing this solvent with petroleum ether, the triacetate separated in large, colorless prisms, m.p. 87–88° (1.5 g., 73%). Recrystallization did not change the m.p.

Anal. Calcd. for $C_{14}H_{16}O_6$ (280.27): C, 59.99; H, 5.75. Found: C, 59.76; H, 5.90.

5-Hydroxy-2,3-dimethyl-1,4-quinone.—A solution of 1.5 g. of the above triacetate in 50 ml. of methanol was swept with oxygen-free nitrogen for 1 hr. and then treated with an oxygen-free mixture of 12 ml. of 25% sodium hydroxide and 50 ml. of water. After a 20-min. period for hydrolysis, 12 ml. of concd. hydrochloric acid was added, followed by 12 ml. of 1 *N* ferric chloride–hydrochloric acid. The solution was then extracted six times with ether and the combined extract was washed with saturated salt solution and extracted with bicarbonate solution. The deep red extract on acidification became clear yellow, and addition of salt to the point of saturation caused separation of the yellow hydroxyquinone. This was extracted with ether and the dried extract on evaporation at reduced pressure afforded 0.9 g. of yellow powder (theory, 0.8 g.). This was dissolved in ether and the solution filtered from a little white solid, diluted with petroleum ether and concentrated to the point of saturation. The hydroxyquinone crystallized in small yellow prisms, m.p. 115–116°; recrystallized 116–117°. The solutions in alkaline buffers are red; λ^{anion} 490 mμ (E_{mol} 1590). The average of three pE determinations[12] was 3.71 ± 0.015. For 2-hydroxy-1,4-naphthoquinone, pE = 3.17 ± 0.08.

Anal. Calcd. for $C_8H_8O_3$ (152.14): C, 63.15; H, 5.30. Found: C, 62.87; H, 5.39.

5-Methoxy-2,3-dimethyl-1,4-quinone was obtained by reaction of the hydroxy compound with diazomethane. It is readily soluble in methanol but crystallizes from this solvent at 5° in bright yellow needles, m.p. 110–111° (unchanged on recrystallization). The ether is stable to hot aqueous bicarbonate solution but readily hydrolyzed by hot sodium carbonate solution. An identical product was obtained by Fischer esterification.

Anal. Calcd. for $C_9H_{10}O_3$ (166.17): C, 65.05; H, 6.07. Found: C, 65.09; H, 5.96.

2,5-Dimethyl-1,3.4-triacetoxybenzene (V).—A suspension of 2 g. of the quinone in 10 ml. of acetic anhydride was treated with 0.5 ml. of boron fluoride etherate, when the

solid soon dissolved. After standing overnight, water was added and the solid that separated was collected; yield 4.07 g. (99%). m.p. 105–107°. Crystallization from methanol–water (3.75 g., 91%) and then from benzene–ligroin gave masses of prisms, m.p. 106–107°.

Anal. Calcd. for $C_{14}H_{16}O_6$ (280.27): C, 59.99; H, 5.75. Found: C, 59.71; H, 5.61.

6-Hydroxy-2,5-dimethyl-1,4-quinone (VI).—This quinone is sensitive and easily destroyed and the process of saponification in methanol–water used for the isomer led to unsatisfactory final product. Omission of methanol improved the results. A suspension of 1 g. of V in 25 ml. of water was swept free of oxygen, 8 ml. of 25% sodium hydroxide solution was added and the mixture was warmed until the triacetate had dissolved and hydrolysis was judged to be complete. Then 8 ml. of concd. hydrochloric acid was added, the solution was filtered, and 8 ml. of 1 *N* ferric chloride–hydrochloric acid was added. The yellow microcrystalline hydroxyquinone that separated was collected after ice cooling; yield 0.5 g., m.p. 140–141°. The substance decomposed on attempted crystallization from water; it is very soluble in cold petroleum ether and could not be crystallized satisfactorily from this solvent. Hence the product as prepared was submitted for analysis.

Anal. Calcd. for $C_8H_8O_3$ (152.14): C, 63.15; H, 5.30. Found: C, 63.55; H, 5.40.

The quinone dissolves in alkaline buffers with a purple color, and the solution absorbs at λ 530 mμ (E_{mol} 1970). A mixture of 100 mg. of the hydroquinone triacetate V, 50 ml. of water and 1 ml. of concd. hydrochloric acid was refluxed for 1 hr. cooled, treated with 3 ml. of ferric chloride solution, extracted with ether three times, and then extracted into a *p*H 10.4 buffer; the extinction coefficient at 530 mμ indicated 92% recovery.

A solution of the hydroxyquinone in methanol was treated with boron fluoride etherate and let stand overnight, diluted and extracted with ether. Bicarbonate extracted the pigment completely from the ether, and hence Fischer esterification had not occurred.

5-Methoxy-2-methyl-1,4-quinone[18] was prepared starting with 5-methyl-1,2,4-triacetoxybenzene (m.p. 112–114°), obtained by Thiele reaction on *p*-toluquinone and three crystallizations from methanol in 47% yield. A mixture of 14.1 g. of triacetate, 100 ml. of water and 15 ml. of concd. hydrochloric acid was boiled in an open flask to allow acetic acid to distil. In 10–15 min. the oil had all dissolved. Boiling was continued for 2 hr., with addition of fresh water as required until near the end, when the volume was reduced to about 50 ml. The nearly colorless solution was cooled and 110 ml. of 1 *N* ferric chloride–hydrochloric acid was added. 5-Hydroxy-2-methyl-1,4-quinone[9] separated in bright yellow crystals and was washed with water and dried at room temperature in the dark. The yield was 6.6 g. (82%), m.p. 150–155° dec. Quinone of this purity can be crystallized nicely from a small volume of petroleum ether; the substance is very sensitive to heat and light.

2,3-Dimethyl-1,4-quinone–2,3-Dimethylbutadiene (VII).—A solution of 2 g. of the quinone and 1.5 g. of the diene[19] in 5 ml. of acetic acid was let stand at 25–27° for 20 hr., when slender, long, white needles had separated. This material was collected (1.2 g., m.p. 103–104°) and the mother liquor on dilution with water afforded a second crop of 1.9 g., m.p. 102–104°; yield 3.1 g. (97%). Crystallization from methanol–water gave needles. m.p. 103–104°.

Anal. Calcd. for $C_{14}H_{18}O_2$ (218.28): C, 77.03; H, 8.31. Found: C, 76.87; H, 8.20.

The adduct is very slowly volatile with steam. It was recovered unchanged after treatment with pyridine and acetic anhydride for 2 hr. at room temperature.

5,8-Dihydro-2,3,6,7-tetramethyl-1,4-naphthohydroquinone (VIIIa).—A solution of 0.65 g. of VII in 4 ml. of acetic acid was treated with 2 ml. of water and 0.2 ml. of concd. hydrochloric acid and heated on the steam-bath for 30 min., when a stiff paste of white solid had formed. This was diluted and the product collected and washed with water. The yield of crystalline white product was 0.6 g., m.p. 266–267°. The substance is sparingly soluble in methanol or acetone and tends to become discolored on attempted crystallization from mixtures containing water. An analytical sample was prepared by adding a drop of hydrochloric acid to a hot solution of 200 mg. of pure adduct in 2 ml. of acetic acid and 1 ml. of water. The hydroquinone separated in excellent flat needles, m.p. 265–266°.

Anal. Calcd. for $C_{14}H_{18}O_2$ (218.28): C, 77.03; H, 8.31. Found: C, 76.92; H, 8.23.

The **diacetate** crystallized from methanol in flat needles, m.p. 190–191°.

Anal. Calcd. for $C_{18}H_{22}O_4$ (302.36): C, 71.50; H, 7.33. Found: C, 71.45; H, 7.16.

5,8-Dihydro-2,3,6,7-tetramethyl-1,4-naphthoquinone (IX).—A solution of 334 mg. of the adduct VII in 15 ml. of methanol was treated at room temperature with 4 ml. of 1 *N* ferric chloride–hydrochloric acid and 0.4 ml. of concd. hydrochloric acid. In a few minutes long, bright yellow needles began to appear; yield 248 mg., m.p. 147–149° dec. A recrystallized sample (methanol) melted at 150–152° dec., with some previous softening. The point of decomposition is not sharp or characteristic.

Anal. Calcd. for $C_{14}H_{16}O_2$ (216.27): C, 77.75; H, 7.46. Found: C, 77.74; H, 7.49.

In an earlier experiment the hydroquinone (1.3 g.) VIIIa was oxidized in hot acetic acid solution (100 ml.) with sodium nitrite[10] at 100°, but analysis showed that the product (m.p. 147–149°) was largely the naphthoquinone X.

2,3,6,7-Tetramethyl-1,4-naphthoquinone (X).—A solution of 1 g. of IX and 2 g. of sodium dichromate dihydrate in 50 ml. of acetic acid was warmed on the steam-bath for 10 min. and diluted to the point of saturation. The naphthoquinone separated in bright yellow blades, m.p. 166–167°, yield 0.92 g. The substance is sparingly soluble in methanol, more soluble in acetic acid (forms yellow prisms). Recrystallization did not change the m.p.

Anal. Calcd. for $C_{14}H_{14}O_2$ (214.25): C, 78.48; H, 6.54. Found: C, 78.36; H, 6.58.

Identification of the Quinones of Gonyleptidine.—The aqueous gonyleptidine extract processed was several months old but had been stored in the frozen state except during the period of air-express shipment from Montevideo. It was a clear yellow solution of *p*H 4 and volume about 43 ml. It was saturated with sodium chloride (13 g.), extracted with ether, and the aqueous layer was drawn off and re-extracted with ether. The combined ethereal solution was filtered through anhydrous sodium sulfate and the bulk of the solvent removed by flash distillation on the steam-bath. When nearly all the ether had distilled the flask was removed and chilled, and last traces of solvent were removed by evacuation at the water pump. The residual yellow oil weighed 115 mg. and when chilled at 5° crystallized in long, slender needles. The pigment was slightly darker than the clear bright yellow material that can be obtained by distillation from the frozen state. The extinction coefficient at λ 255 mμ (water) was $E^{1\%}$ = 1280, that found repeatedly in Montevideo for distilled fresh extract was 1400.

The total 115 mg. of material was employed for preparing the spectrographic sample and was then recovered by the above process, dissolved in 2 ml. of acetic acid, and treated with 0.2 ml. of 2,3-dimethylbutadiene. After standing in the dark at 25–27° for 20 hr., the still yellow solution was taken up in ether and shaken once with saturated salt (NaCl) solution, which was removed. Successive small portions of 5% sodium bicarbonate solution were then shaken with the ether and the exhausted liquor let accumulate. When all the acetic acid had been neutralized, saturated salt solution was added before withdrawal of the aqueous layer. A solution of 300 mg. of sodium hydrosulfite in 2 ml. of water was added, along with 15 ml. of saturated salt solution, and the mixture was shaken until both layers were colorless; the aqueous layer was then discarded. At this point the ethereal solution should be in a funnel that allows no more than about 20% air space. A solution of 25 mg. of sodium hydrosulfite in 5 ml. of water was added, then 3 ml. of 10% sodium hydroxide, and the mixture was shaken thoroughly without removing the stopper. The alkaline layer, which remained colorless, was drawn off into a flask containing 2 ml. of concd. hydrochloric acid and 5 ml. of water, and the ether was rinsed with small portions of water to remove the alkaline liquor as fully as possible. A further

(18) B. D. W. Luff, W. H. Perkin, Jr., and R. Robinson, *J. Chem. Soc.*, **97**, 1137 (1910).

(19) Dajac Laboratories diene, freshly distilled from the antioxidant.

extraction with 3 ml. of 10% alkali and 5 ml. of water required no additional hydrosulfite and apparently removed little further hydroquinones. The neutral ethereal solution was shaken with salt solution, filtered through sodium sulfate, and evaporated. The residual, nearly colorless, solid weighed 106.5 mg. and melted at 100–102°. Crystallization from methanol–water gave 76 mg. of material, m.p. 102–103°, identified by the melting point (103–104°) of a mixture with the **2,3-dimethyl-1,4-quinone—2,3-dimethylbutadiene adduct.** For confirmation, the material was converted by acid isomerization, oxidation with nitrous acid and then with dichromate, and crystallization from methanol, into **2,3,6,7-tetramethyl-1,4-naphthoquinone**, m.p. 166–167°, mixed m.p. 166–167°.

The acidified solution of the aqueous alkaline extract was treated with 5 ml. of a solution 1 *N* in both ferric chloride and hydrochloric acid, and sodium chloride was added to the point of saturation. Extraction with ether gave a bright yellow oil that was treated with 1 ml. of acetic anhydride and 0.05 ml. of boron fluoride etherate and let stand at 25–27° for 2 hr. The still yellow solution was rinsed with a little ether into a 50-ml. distilling flask, 25 ml. of water was added and the mixture distilled. Ether came over first, followed by a yellow aqueous solution, and distillation was stopped when the solution remaining in the boiling flask was colorless. This residual solution was neutralized by addition of successive small portions of solid sodium bicarbonate, then salt was added until no more would dissolve, and the solution was extracted with ether. Evaporation of the dried solution gave 14.5 mg. of nearly colorless, crystalline product. This was dissolved in 1:1 ether–petroleum ether and the solution was clarified with Norit and then evaporated, with repeated addition of petroleum ether until diethyl ether was largely removed. After standing for some time at 5°, a small mass of prisms grew on the carborundum boiling stone, m.p. 102–103°; mixed m.p. with 2,5-dimethyl-1,3-triacetoxybenzene (m.p. 105–106°), 104–105°. The infrared spectrum was superposable on that of a synthetic sample.

The yellow aqueous steam distillate when suitably diluted showed an absorption maximum at 260 mμ (in water) with an extinction coefficient corresponding to 11.9 mg. of 2,3,5-trimethyl-1,4-quinone. The infrared spectrum corresponded exactly in all regions with that of synthetic quinone. The quinone was recovered by saturation with salt and ether extraction and treated with zinc dust, acetic anhydride and triethylamine for 10 min. at room temperature. The product, recovered after hydrolysis, washing with mineral acid and then bicarbonate, was taken up in ether and the solution was treated with petroleum ether and boiled down to the point of saturation. A white powder separated, m.p. 165–168°, and when recrystallized from benzene formed three small prisms, m.p. 166–168°; a mixture with **2,3,5-trimethylhydroquinone** melted at 166–169° and remelted at 165–168°. The material recovered from the mother liquors was heated with acetic anhydride and pyridine for 15 min. on the steam-bath and the neutral product crystallized from petroleum ether at 5°. It formed a small rosette of needles, m.p. 99°; a mixture with **2,3,5-trimethylhydroquinone diacetate** (m.p. 104–105°) melted at 100–102°.

The above procedure was worked out in trials on a synthetic mixture that, fortuitously, proved comparable in amount and proportions to the sample of gonyleptidine subsequently processed: 100 mg. of 2,3-dimethyl-1,4-quinone, 25 mg. each of 2,5-dimethyl-1,4-quinone and 2,3,5-trimethyl-1,4-quinone. Derivatives of each of the three components were isolated by the above procedure and identified after crystallization. The yields of uncrystallized products appear to afford a reliable indication of content and were as follows: 2,3-dimethyl-1,4-quinone—2,3-dimethylbutadiene, 150 mg. (94%); 2,5-dimethyl-1,3,4-triacetoxybenzene, 51.5 mg. (64%); 2,3,5-trimethylhydroquinone, 22 mg. (78%). On the assumption that the recovery was the same in the processing of gonyleptidine as in the processing of the synthetic mixture, the 115 mg. of gonyleptidine processed is estimated to have contained 71 mg. of 2,3-dimethyl-1,4-quinone, 11 mg. of 2,5-dimethyl-1,4-quinone and 15 mg. of 2,3,5-trimethyl-1,4-quinone.

CAMBRIDGE, MASS.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, OREGON STATE COLLEGE]

The Synthesis of Certain O-Substituted Derivatives of Hydroxyguanidine and Hydroxybiguanide[1]

BY DAVID D. NYBERG ABD BERT E. CHRISTENSEN

RECEIVED SEPTEMBER 12, 1955

The syntheses of several oxygen (O) substituted alkyl derivatives of hydroxyguanidine and hydroxybiguanide which are of biological interest are described. These compounds were prepared from the corresponding α-alkylhydroxylamines using methylisothiourea sulfate to introduce the guanidine group and cyanoguanidine with cupric sulfate to produce the biguanides.

Various compounds possessing the basic structure of urea, thiourea, and/or guanidine have been shown to have biological activity. Canavanine, a close structural analog of arginine, recently has been shown to be a growth inhibitor of various bacterial species, and of both the Lee influenza and mouse encephalomyelitis viruses.[2] Other compounds such as paludrine or 1-(*p*-chlorophenyl)-5-isopropylbiguanide hydrochloride, 1-(*p*-chlorophenyl)-biguanide hydrochloride, L-arginine, various aldehyde semicarbazones, and guanylureas are of biological interest and possess the structural units listed above.

In conjunction with a study of the inhibitory properties of compounds of these types, the synthesis of certain O-substituted derivatives of hydroxyguanidine and hydroxybiguanides was undertaken. Many of these have not been prepared previously and such preparations are described in this paper.

The starting materials for these series of compounds were the corresponding alkoxyurethans and α-alkylhydroxylamines. All of these had previously been prepared by other workers,[3-4] with the exception of 3-methylbutoxyurethan (isoamoxyurethan) and α-3-methylbutylhydroxylamine (α-isoamylhydroxylamine). The method is, in general, the same for all of these compounds with the exception of α-methylhydroxylamine whose syn-

(1) These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and Oregon State College. Published with the approval of the Monographs Publication Committee, Oregon State College as Research Paper No. 284, School of Science, Department of Chemistry.

(2) K. S. Pilcher, K. F. Soike, V. H. Smith, F. Trosper and B. Folston, *Proc. Soc. Exp. Biol. Med.*, **88**, 79 (1955).

(3) C. H. Hecker, *Am. Chem. J.*, **50**, 445 (1913).

(4) (a) C. H. Andrews, H. King and J. Walker, *Proc. Roy. Soc.* (*London*), **B133**, 43–45 (1946); (b) A. T. Fuller and H. King, *J. Chem. Soc.*, 963 (1947).