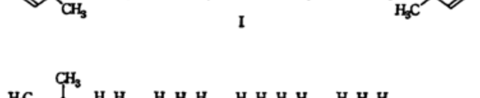
*Renieratene, a New Carotenoid Containing Benzene Rings, Isolated from a Sea Sponge**

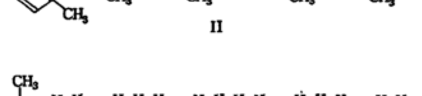
By Masaru YAMAGUCHI

(Received March 5, 1958)

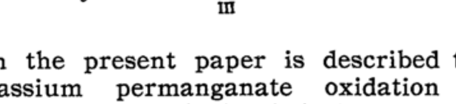
With regard to the chemical constitution of renieratene[1], a carotenoid pigment isolated from a sponge "*Reniera japonica*", it has previously been shown from the result of the chromic acid oxidation, by which crocetin dialdehyde has been isolated besides other products, that renieratene is a derivative of tetramethyl-octadeca-nonaene[2]. Shortly afterwards the author has elucidated the chemical constitution of isorenieratene (I), and it has become clear that isorenieral which is the degradation product of the former, has the constitution indicated by II[3]. As isorenieral is also one of the degradation products of renieratene, it is clear that renieratene can be expressed by the partial formula III. Therefore the remaining problem in the chemical constitution of renieratene is the elucidation of R-group in the formula III. From the molecular formula of renieratene, $C_{40}H_{48}$, and the number of double bonds, 15, it can be concluded that the R-group has the formula C_9H_{11}–, and that it contains one ring and three double bonds. Furthermore, it can be inferred from the analogy with another terminal group, that the ring may be a benzene ring.



I



II



III

In the present paper is described the potassium permanganate oxidation of renieratene, by which whole its constitution has been clarified.

The oxidation products were separated into some neutral and acidic fractions by the ordinary method. From the neutral

---

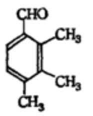
* "Pigments of Marine Animals", VI. V of this series, This Bulletin, **31**, 51 (1958).

[1] T. Tsumaki, M. Yamaguchi and T. Tsumaki, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **75**, 297 (1954); M. Yamaguchi, This Bulletin, **30**, 111 (1957).

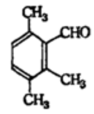
[2] M. Yamaguchi, ibid., **30**, 979 (1957).

[3] M. Yamaguchi, ibid., **31**, 51 (1958).

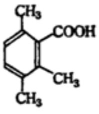
fractions a considerable amount of 2,3,4-trimethylbenzaldehyde (IV) was obtained in the form of semicarbazone. The confirmation was done by the mixed melting point determinations of the semicarbazone and 2,4-dinitrophenylhydrazone with the authentic samples and further by the oxidation into 1,2,3,4-benzene-tetracarboxylic acid. As another neutral constituent, 2,3,6-trimethylbenzaldehyde (V) which was fairly reasonably expected, was obtained in the form of 2,4-dinitrophenylhydrazone by the chromatographic separation, from the mother liquor of the above semicarbazone. From the petroleum ether soluble part of the acidic fraction, a small quantity of 2,3,6-trimethylbenzoic acid (VI) was isolated in the form of its 4-phenylazophenacyl ester. However the main part of the acidic fraction consisted of an acid of m. p. 205~220°, which gave 1,2,3,4-benzene-tetracarboxylic acid, on oxidation. As the acid of m. p. 205~220° could not be purified effectively by recrystallization, it was converted into 4-phenylazophenacyl ester. The main product thus obtained after the chromatographic separation was a di-ester of 2,4-dimethyl-benzene-1,3-dicarboxylic acid (VII).



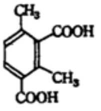
IV



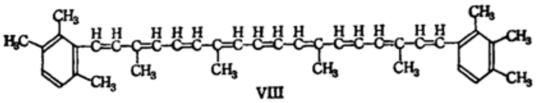
V



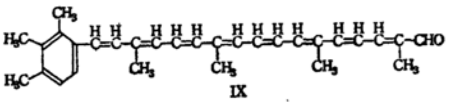
VI



VII



VIII



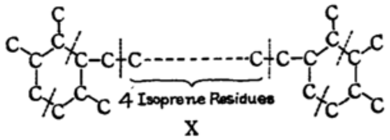
IX

These results decidedly show that besides 2,3,6-trimethylphenyl group as one terminal group, 2,3,4-trimethylphenyl group must exist as another terminal group which has been expressed by R-group in III. Therefore the structural formula of renieratene can be given by VIII. This formula can explain all its properties or behaviors hitherto observed[1,2].

Renieral, which, as well as isorenieral, is a degradation product of the mild oxidation of renieratene[2], can therefore be considered to have a 2,3,4-trimethylphenyl group, and can be expressed by the formula IX.

It has already been pointed out that for the explanation of the spectral properties of isorenieratene, it is necessary to suppose the steric hindrance caused by the two methyl groups which are standing at the two neighboring positions of the polyene chain in benzene ring[3]. In the case of renieratene, the position of the absorption maxima can also be accounted for by the same effect. The difference between the wavelength of the longest wavelength maximum of diphenyltetramethyl-octadeca-nonaene[4], 541 mμ in carbon disulfide, and that of isorenieratene, 520 mμ in the same solvent, is ca. 21 mμ. The effect caused by only one 2,3,6-trimethylphenyl group can therefore be considered to be a half, i. e. about 10 mμ. Renieratene has one 2,3,6-trimethylphenyl group and one 2,3,4-trimethylphenyl group. As the latter possesses only one methyl group at the *o*-position to the polyene chain, the steric effect may be much smaller than that caused by the former. Therefore renieratene will possess the longest wavelength maximum at ca. 10 mμ shorter wavelength position than that of unsubstituted diphenyltetramethyl-octadecanonaene, i. e. at 531 mμ. This value shows good agreement with the observed value, 532 mμ, of renieratene.

Renieratene can be considered to be resolved into isoprene unites. However, as indicated in X, the modes of binding of isoprene residues in the terminal groups of renieratene are considerably different from those in the carotenoids of plant-occurence, in which isoprene residues are arranged top-to-tail. Therefore, in these respect also, renieratene can be regarded as a carotenoid of a new type.



X

Experimental

Renieratene (200 mg.) dissolved in pure benzene (90 cc.) and potassium permanganate (2.7 g.) and sodium carbonate (2.7 g.) dissolved in water (100 cc.) were stirred efficiently at the room temperature. After 8 hours the color of the benzene layer completely disappeared. The excess of permanganate was reduced with the solution of sodium hydrogen sulfite, the precipitates were

---

[4] C. H. Eugster, C. Garbers and P. Karrer, *Helv. Chim. Acta*, **35**, 1179 (1952).

filtered and washed several times with hot benzene and water. The filtrate and washings were combined and treated as below.

2,3,4-Trimethylbenzaldehyde.—The benzene layer of the above product was separated, washed with water, dried with sodium sulfate, and concentrated under diminished pressure. The oily residue was dissolved in ethanol (10 cc.) and treated with the solution of semicarbazide hydrochloride (40 mg.) and sodium acetate (80 mg.) in water (2 cc.). After 24 hours the precipitates were collected, and the recrystallization from ethanol gave 2, 3, 4-trimethylbenzaldehyde semicarbazone in colorless plates, m. p. 240～242°, (16 mg.).

Anal. Found: C, 64.47; H, 7.43. Calcd. for $C_{11}H_{15}ON_3$: C, 64.36; H, 7.37%.

The semicarbazone (7 mg.), potassium permanganate (45 mg.) and sodium carbonate (45 mg.) were mixed and finely ground, and after the addition of water (3 cc.) heated on the steam bath for 12 hours with occasional additions of a small amount of potassium permanganate. The precipitates were dissolved by the addition of sodium hydrogen sulfite solution and diluted sulfuric acid, and the solution was extracted many times with ether. Ether was evaporated and the residue, recrystallized from water, yielded 1, 2, 3, 4-benzene-tetracarboxylic acid in colorless plates (2 mg.), m. p. 215～236°, which was identified by the light absorption curve and mixed melting point determination with the authentic sample.

The semicarbazone gave 2,4-dinitrophenylhydrazone in red needles, m. p. 227～229°, when treated with 2, 4-dinitrophenylhydrazine in ethanolic sulfuric acid. The semicarbazone and the hydrazone exhibited no melting point depression when mixed with the authentic samples derived from the synthetic 2, 3, 4-trimethylbenzaldehyde. (It was synthesized from 4-bromohemimellithene and ethyl orthoformate by Grignard reaction[5]. Semicarbazone, colorless plates from ethanol, m. p. 240～242°. *Anal.* Found: C, 64.28; H, 7.21. Calcd. for $C_{11}H_{15}ON_3$: C, 64.36; H, 7.37%. 2, 4-dinitrophenylhydrazone, red needles from benzene-ethanol, m. p. 229°. *Anal.* Found: C, 58.77; H, 5.09. Calcd. for $C_{16}H_{16}O_4N_4$: C. 58.53; H, 4.91%.)

2,3,6-Trimethylbenzaldehyde.—The mother liquor of the above semicarbazone was treated with 2, 4-dinitrophenylhydrazine and sulfuric acid in ethanol. The precipitates (31 mg.) were recrystallized from benzene-ethanol and adsorbed on alumina (column, 2×15 cm.; developer, benzene), forming two main zones. The upper brown zone gave red needles of 2, 3, 4-trimethylbenzaldehyde-2′, 4′-dinitrophenylhydrazone (11 mg.), m. p. 225～228°, when recrystallized from benzene-ethanol.

Anal. Found: C, 58.62; H, 5.18. Calcd. for $C_{16}H_{16}O_4N_4$: C, 58.53; H, 4.91%.

The lower orange zone afforded 2, 3, 6-trimethyl benzaldehyde-2′, 4′-dinitrophenylhydrazone in red needles (13 mg.), m. p. 219°.

Anal. Found: C, 58.88; H, 4.94. Calcd. for $C_{16}H_{16}O_4N_4$: C, 58.53; H, 4.91%.

2,3,6-Trimethylbenzoic Acid.—The aqueous solution separated from benzene layer containing neutral fractions was concentrated under vaccum to ca. 4 cc., acidified with sulfuric acid (20%), and extracted with ether (total, 50 cc.). Ether was evaporated and the residue was extracted with petroleum ether (3 cc.). The petroleum ether solution was evaporated and the oily residue was neutralized with the solution of sodium hydroxide (20.6 cc., 0.0143 N) using phenolphthalein. The indicator was removed by the ether extraction, the solution was evaporated to dryness and the residue was heated under reflux with 4-phenylazophenacyl bromide[6] (80 mg.) in ethanol (3 cc.) for 30 minutes. The product was dissolved in benzene (10 cc.), washed with water, dried, and chromatographed on alumina (column, 2×20 cm.; developer, benzene) giving seven zones. The third orange zone from the top afforded faint red needles (ca. 1 mg.), m. p. 165～169°, when recrystallized from benzene-ethanol. It seemed to be an impure ester of 2, 4-dimethylbenzene-1, 3-dicarboxylic acid described below. The sixth orange zone afforded red needles of 4′-phenylazophenacyl-2, 3, 6-trimethylbenzoate (2.3 mg.), m. p., 160～162°, when the residue of the eluate was recrystallized twice from benzene-ethanol. Mixed m. p. 160～162°.

Anal. Found: N, 7.28. Calcd. for $C_{24}H_{22}O_3N_2$: N, 7.25%.

2,4-Dimethylbenzene-1,3-dicarboxylic Acid.—The colorless residue of the above petroleum benzine extraction was recrystallized from water giving colorless solid (12 mg.), m. p. 205～220°. As it was unsuccessful to obtain pure substance by recrystallization, a part of the sample (5 mg.) was oxidized with potassium permanganate (35 mg.) and sodium carbonate (35 mg.) in a way as described in the oxidation of semicarbazone, yielding a small quantity of 1, 2, 3, 4-benzene-tetracarboxylic acid in colorless plates, m. p. 215～230°, mixed m. p. 215～230°.

The remaining part (ca. 6 mg.) was neutralized with a sodium hydroxide solution and after the removal of the indicator the solution was evaporated. The residue was refluxed with 4-phenylazophenacyl bromide (20 mg.) in ethanol (3 cc.) for one hour. When chromatographed on alumina (column, 2×15 cm.; solvent, benzene) the precipitates gave a main orange zone besides several minor zones. The main zone was eluted with benzene containing methanol and the residue of the eluate was recrystallized from benzene-ethanol, yielding di-(4′-phenylazophenacyl)-2, 4-dimethylbenzene-1,3-dicarboxylate in fine red needles (5 mg.), m. p. 169～172°.

Anal. Found: C, 71.15; H, 4.75. Calcd. for $C_{38}H_{30}O_6N_4$: C, 71.46; H, 4.74%.

---

[5] The aldehyde has been reported to be synthesized from hemimellithene by Gattermann-Koch's reaction, but no characterizations has been described. H. A. Smith and J. A. Stanfield, *J. Am. Chem. Soc.*, **71**, 81 (1949).

[6] S. Masuyama, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zassi*), **71**, 402 (1950).

The ester exhibited no melting point depression when mixed with the authentic sample (prepared from synthetic acid[3]; red needles, m. p. 172°. *Anal*: C, 71.12; H, 4.83%.).

The author wishes to express his thanks to Professor Tokuichi Tsumaki for his exceedingly kind directions throughout this work. The author also thanks Mr. Michio Shido and Miss Sachiko Indo for microanalysis. The cost of this work has been partially covered by the Scientific Research Grant of Ministry of Education.

Department of Chemistry
Faculty of Science
Kyushu University
Hakozaki, Fukuoka