tion about 20 mg. of a solid was isolated from 4000 liters of urine that supported half-maximum growth of *C. fasciculata* at a concentration of 0.05 millimicrograms per ml. The name biopterin is suggested for this substance. The material precipitated as pale yellow spheres from hot water and decomposed without melting at 250–280°. It was optically active, $[\alpha]^{25}D$ −50 (0.1 *N* HCl; *C*, 0.4). It was insoluble in the common organic solvents, slightly soluble in water, soluble in dilute acid and alkali. Analysis calculated for $C_9H_{11}N_5O_3$: C, 45.6; H, 4.64; N, 29.5; mol. wt., 237. Found on two separate samples, C, 42.8, 43.6; H, 5.00, 4.84; N, 29.3; S, negative.

The ultraviolet absorption spectrum of biopterin was typical of 2-amino-4-hydroxy-alkylpteridines with maxima at 254 mμ and 363 mμ in 0.1 *N* NaOH. Assuming biopterin has the same molecular extinction as 2-amino-4-hydroxy-alkylpteridines, a molecular weight of 282 was calculated from the ultraviolet absorption. The infrared spectrum measured on a KBr pellet showed strong bands at 3650–3450, 3310, 2975–2930, 1695–1680, 1538, 1490, 1418, 1370, 1295, 1245, 1173, 1130, 1063 and 823 cm.$^{-1}$.

Biopterin titrated with periodate consumed 1.7 and 4.5 oxidation equivalents per molecular weight of 237 at *p*H 2 and 8.5, respectively. Formaldehyde, formic acid and ammonia were not detected in the oxidation mixtures. Upon adjusting the oxidation mixtures to *p*H 5, yellow precipitates came down, and the ultraviolet absorption spectra of these indicated that the material from the *p*H 2 oxidation was 2-amino-4-hydroxy-6-formylpteridine and the material from the *p*H 8.5 oxidation was 2-amino-4-hydroxy-6-carboxypteridine. Thus biopterin appears to consume about two more periodate oxidation equivalents in alkali by the pteridine aldehyde being oxidized to the corresponding acid. From these data and studies on models it is concluded the structure of biopterin is 2-amino-4-hydroxy-6-(1,2-dihydroxypropyl)-pteridine.

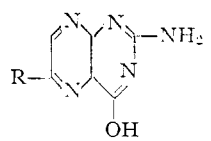
The microbiological response to biopterin is now obtained in the absence of added PGA.[3] The involvement of PGA in the metabolism of *C. fasciculata* was demonstrated indirectly, however, by adding an inhibitory amount of 2,4-diamino-5-*p*-chloro-phenoxy-6-ethylpyrimidine (I) to the culture medium. Under these conditions growth was obtained by adding sufficient PGA to counteract I only if biopterin was present (Table 2). The data of Tables I and II illustrate that *C. fasciculata* required both PGA and a pteridine for growth, implying that these structurally-related substances have independent metabolic functions for this organism.

We are indebted to Mrs. L. H. Smith for some of the microbiological assays and to Dr. S. H. Hutner, Haskins Laboratories, New York, N. Y. for his suggestions concerning the culturing of the test organism.[4]

TABLE I

THE EFFECT OF PGA ON THE GROWTH RESPONSE OF *Crithidia fasciculata* TO URINE OR TO CERTAIN 6-SUBSTITUTED PTERIDINES

Additions per tube (2.5 ml.) final strength medium[a]	PGA Additions to medium: None	10 mγ	100 mγ	1000 mγ
	Optical Density			
None	0.02	0.05	0.11	0.88
0.001 ml. urine	0.02	0.64		
0.01 ml. urine	0.05	0.98		
0.1 ml. urine	0.37	1.05		



R = CH_2OH[b]	10 mγ	0.02	0.25	0.65	0.87
	100 mγ	0.07	0.58	0.81	0.88

[a] Test conditions were those described by Nathan and Cowperthwaite[2] except that the adenosine was replaced by 100γ adenine, guanine and uracil per tube and the assay was carried out in slanted test tubes rather than flasks. [b] Microbiological findings with other pteridines tested with 10 mγ PGA in the medium were as follows: When R = H, OH or COOH, no activity at 10γ/tube, when R = CHO or CH_3, 0.3 to 0.1 as much activity as when R = CH_2OH (see table). 2-Amino-4-hydroxy-5-formyl-6-methyltetrahydropteridine, the methylpteridine moiety of leucovorin, was inactive at 10γ/tube.

TABLE II

TOXICITY OF 2,4-DIAMINO-5-*p*-CHLOROPHENOXY-6-ETHYLPYRIMIDINE FOR GROWTH OF *Crithidia fasciculata*: REVERSAL BY PGA[a]

Biopterin	PGA	2,4-Diamino-5-*p*-chlorophenoxy-6-ethylpyrimidine (1): 0 γ	1 γ	3 γ	10 γ
		Optical Density			
....	....	0.03	0.02	0.01	0.03
10 mγ	....	.43	.02	..	..
100 mγ	....	.46	.09	..	..
....	10 mγ	.03	..	..	..
....	100 mγ	.02	..	..	..
100 mγ	10 mγ	.49	.49	.04	..
100 mγ	100 mγ	.46	.45	.38	.03

[a] Test conditions as in footnote to Table 1; 2.5 ml. final volume.

(2) H. A. Nathan and J. Cowperthwaite, *Proc. Soc. Exptl. Biol. Med.*, **86**, 117 (1954).

(3) Within the past year the test organism apparently "lost" its nutritional requirement for preformed folic acid. Factors responsible for this change are unknown.

(4) ADDENDUM.—After this Communication was submitted for publication, the Editors informed us that Forrest and Mitchell have isolated and characterized what appears to be the same pteridine from wild type *Drosophila*.

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RECEIVED MARCH 24, 1955

BOND ORDER—LENGTH RELATIONSHIPS

Sir:

The importance of establishing reliable bond order-length relationships for as many atom-pairs as possible is generally recognized. There are several ways of defining bond order (w). Examples are the original method suggested by Pauling, Brockway and Beach, and the more recent method of molecular orbitals. Bond order length curves have been usually constructed by plotting

bond order against bond length, and attempts have been made to give these curves a theoretical or semi-theoretical basis. Empirical equations connecting these two internuclear properties have been suggested. It is assumed now, as in the past, that single, double and triple bonds have their conventional orders, and second order refinements are properly neglected.

For the hydrogen molecule, r_e = 0.74 Å., n = I, and for the hydrogen molecule ion, r_e = 1.06 Å., n = 0.5. Also, $(1/r_e^2)$ = 1.826 and 0.890, which figures suggest that bond order and $1/r_e^2$ have some connection. This possibility may be tested rigorously for carbon–carbon bonds. The carbon–carbon bond length in ethane is 1.543 Å., in ethylene 1.353 Å., and in acetylene 1.207 Å. Also, using the rotational Raman spectrum, Stoicheff[1] has found that the C–C distance in benzene is 1.397 Å., and by the molecular orbital method; Coulson[2] has evaluated the C–C bond order in this molecule as 1.67. If now bond order is plotted against $1/r_e^2$ an excellent straight line is obtained, having the equation

$$1/r_e^2 = 0.2868 + 0.1334n \quad (1)$$

In Table I, the last column gives values of r_e calculated using equation (I).

TABLE I

n	r_e, Å.	$1/r_e^2$	$(r_e)_c$, Å.
1	1.543	0.4202	1.543
1.67	1.397	0.5121	1.401
2	1.353	0.5462	1.344
3	1.207	0.6868	1.207

For the carbon–nitrogen pair,[3] bond length information is on a lower plane of accuracy. A careful assessment of the data available on the C–N single bond has given the value 1.475 Å., and for the triple bond spectroscopic methods have led to 1.156 Å. Again, theoretical bond orders calculated by the molecular orbital method, and experimental bond lengths are available for the molecules, melamine and pyridine. For melamine, the bond order of each C–N bond is 1.658, and the bond length 1.346 Å., and for pyridine the values are 1.534, and 1.37 Å. Table II gives the data, the equation to the line being;

$$1/r_e^2 = 0.316 + 0.144n \quad (2)$$

TABLE II

n	r_e, Å.	$1/r_e^2$	$(r_e)_c$, Å.
1	1.475	0.460	1.475
1.53	1.37	0.533	1.37
1.66	1.346	0.552	1.35
3	1.156	0.748	1.156

For nitrogen–nitrogen bonds, the single bond distance is known from electron diffraction measurements on hydrazine to be 1.48 Å. The double bond distance in azomethane is 1.24 Å., and the triple bond distance in nitrogen is 1.094 Å. A plot of bond order against $1/r_e^2$ gives an almost perfect straight line having the equation;

$$1/r_e^2 = 0.263 + 0.193n \quad (3)$$

(1) B. P. Stoicheff, *Can. J. Phys.*, **32**, 635 (1954); G. Herzberg and B. P. Stoicheff, *Nature*, **175**, 79 (1955).

(2) C. A. Coulson, *Proc. Roy. Soc.*, **169A**, 413 (1939).

(3) E. G. Cox and G. A. Jeffrey, *ibid.*, **207A**, 110 (1951).

Similar relationships probably exist for carbon–oxygen and carbon–sulfur bonds.

THE UNIVERSITY
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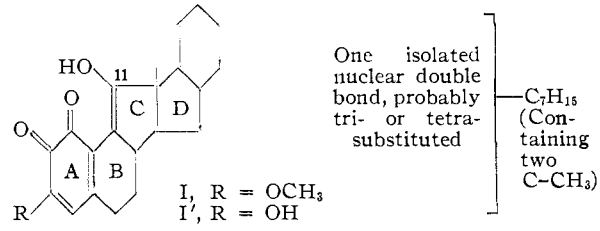
RECEIVED MAY 9, 1955

STRUCTURE OF PRISTIMERIN AND CELASTROL[1]

Sir:

Pristimerin is an antibiotic isolated from *Pristimera indica* and *P. grahami* (*Celastraceae*),[2] which besides being active against the common gram-positive organisms, is characterized by its activity against the *Viridans* group of Streptococci. Comparison of the melting point, ultraviolet and infrared spectra of pristimerin with celastrol monomethyl ether[3] showed that the two compounds were identical.[4] We wish to present structure I for pristimerin and I′ for celastrol as a working hypothesis.



I, R = OCH_3
I′, R = OH

Pristimerin, $C_{30}H_{40}O_4$ (Calcd.: C, 77.55; H, 8.68. Found: C, 77.54; H, 8.87), orange needles, m.p. 219–220°, possesses one methoxyl group and at least three C-methyl groups (Kuhn–Roth: Found 2.5 groups): $\lambda_{infl.}^{alc.}$ 250–255 mμ (log ε 3.90), $\lambda_{min.}^{alc.}$ 305 mμ (log ε 2.65), $\lambda_{max.}^{alc.}$ 423 mμ (log ε 4.05).[5] The reversible nature of color discharge, when reacted upon by reducing agents, *e.g.*, PtO_2–H_2, sodium hydrosulfite, etc., and the similarity in the shape[6] of the ultraviolet spectrum with that of model *o*-quinones, *e.g.*, 3-methoxy-1,2-benzoquinone ($\lambda_{min.}^{alc.}$ 290 mμ, log ε 2.68; $\lambda_{max.}^{alc.}$ 370 mμ, log ε 3.31) suggested that two of the oxygen functions were contained in an *o*-quinonoid arrangement. However, the colorless reductive acetate II, $C_{34}H_{46}O_6$ (Calcd.: C, 74.15; H, 8.42. Found: C, 73.75; H, 8.29), m.p. 252°, $[\alpha]^{22}D$ +54.3 ($CHCl_3$), did not show any conspicuous maxima in the ultraviolet, excepting a shoulder-like peak at 278 mμ (log ε 2.90), which at most could only be attributed

(1) The present studies were commenced in the laboratory of Professor L. F. Fieser, Harvard University.

(2) S. S. Bhatnagar and P. V. Divekar, *J. Sci. Industr. Res.*, **10B**, 56 (1951).

(3) O. Gisvold, *J. Am. Pharm. Soc.*, **28**, 449 (1939); **29**, 12 (1940); **31**, 529 (1942); L. F. Fieser and R. N. Jones, *ibid.*, **31**, 315 (1942); M. S. Schechter and H. L. Haller, THIS JOURNAL, **64**, 182 (1942).

(4) We are indebted to Professor O. Gisvold, University of Minnesota, for kindly furnishing us a sample of celastrol and the monomethyl ether. The probable identity of pristimerin and celastrol methyl ether has been previously noted by Kulkarni and Shah[11] and by Kamat, *et al.*[14]

(5) Though the infrared spectrum has been compared with those of several other *o*-quinones, we have not been able to make satisfactory assignments in the 6 μ region: $\lambda_{max.}^{Nujol}$ 3350 w, 1742 s, 1736 s, 1662 m, 1595 s, 1550 m, 1519 m; $\lambda_{max.}^{CCl_4}$ 3380 w, 1740 s, 1655 w, 1607 s, 1514 m.

(6) The batho- and hyper-chromic shifts encountered in the spectrum of pristimerin in comparison to other *o*-quinones is now attributed to the conjugated double bond.