636. *Pteridine Studies. Part XXVI.*[1] *Acid Catalysis of Michael-type Reactions. Resolution of a Racemic Dipteridinylmethane during Paper Chromatography.*[2]

By Adrien Albert and E. P. Serjeant.

"Substance T," a racemate formed by the reaction of ethyl pyruvate with 4,5-diaminopyrimidine, was resolved into its enantiomers during paper chromatography. The substance, shown to be 7,8-dihydro-6-hydroxy-7-(7-hydroxypteridin-6-ylmethyl)-7-methylpteridine, (I), was formed by the acid-catalysed addition of 7-hydroxy-6-methyl- to 6-hydroxy-7-methyl-pteridine.

Such acid-catalysed Michael-like additions, although unprecedented, were shown to be general by the use of quinazoline as a model receptor, and of 4-methylquinazoline and acetylacetone as model donors. A mechanism is suggested.

When ethyl pyruvate and 4,5-diaminopyrimidine reacted in highly acidic aqueous solutions, 6-hydroxy-7-methyl-(and, in very weak acid, 7-hydroxy-6-methyl-)pteridine were produced in good yield.[3] However, in the intermediate range, pH 1—3, only an amphoteric solid ($C_{14}H_{12}N_8O_2$) of molecular weight about 320 was obtained. This substance, although behaving chemically as a pure species, produced *two* similar spots on paper chromatography.

A larger quantity, chromatographed on a cellulose column, gave two fractions which differed only in optical activity; crystals from the faster-running fraction had the specific rotation $[\alpha]_{546}^{20}$ +180 (±5°), whereas those from the other fraction had −180 (±5°) measured at pH 9·1.

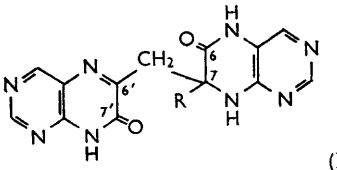
The original racemate, which we shall show is the dipteridinylmethane (I), will be referred to briefly as "Substance T." It was more conveniently prepared by refluxing 6-hydroxy-7-methyl- and 7-hydroxy-6-methyl-pteridine in water. The highest yield (40%) was produced at pH 2·5, and it fell steadily to 10% at pH 0 and 9 (at pH 11 both starting materials were recovered unchanged). Conversely, when "Substance T" was heated with 0·5N-sodium hydroxide, 6-hydroxy-7-methyl- and 7-hydroxy-6-methyl-pteridine were regenerated. These phenomena suggested the occurrence of an acid-catalysed addition, and its reversal by alkali.

It is known[3] that, in cold alkaline solution, 6-(but not 7-)hydroxypteridine readily adds (across the 7,8-position) such Michael reagents as ethyl malonate, ethyl cyanoacetate, and even acetone. This suggested that "Substance T" is formed by the similar addition of 7-hydroxy-6-methylpteridine across the 7,8-position of 6-hydroxy-7-methylpteridine, to give the dipteridinylmethane (I), *viz.*, 7,8-dihydro-6-hydroxy-7-(7-hydroxypteridin-6-ylmethyl)-7-methylpteridine, which has an asymmetric carbon atom at C-7. (In what follows, the Michael-donor molecules will be named as substituents in the Michael-receptors. This small departure from the accepted nomenclature, that the less hydrogenated substance should be the stem, makes for increased clarity in the present study. Similarly, the formulæ of hydroxy-compounds show the predominant oxo-tautomer.) To test this hypothesis, it was necessary to show (*a*) that the addition of carbanions to the C=N bond can be catalysed by acids, (*b*) that the methyl group in 7-hydroxy-6-methylpteridine is activated, and more specifically that it can act as a carbanion donor to the C=N bond in acid solution, (*c*) that 6-hydroxy-7-methylpteridine is a carbanion receptor in acid solution, and (*d*) that "Substance T" can be degraded to fragments which confirm structure (I).

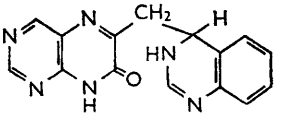
[1] Part XXV, Albert and Clark, *J.*, 1964, 1666.
[2] Preliminary account of this resolution, Albert and Sergeant, *Nature*, 1963, **199**, 1098.
[3] Albert and Reich, *J.*, 1961, 127.

With one possible exception [4] the addition of carbanions, such as acetylacetone to C=C (the Michael reaction [5]) and to C=N,[6,7] requires neutral or alkaline conditions.[3,8] However, we found that to obtain a quantitative yield of the adduct * from 6-hydroxypteridine and acetylacetone a pH of 2·5 was required.

To determine whether the methyl group in 7-hydroxy-6-methylpteridine is an effective carbanion donor in acid solution, it was allowed to react (in aqueous solution at pH 2·5) with the 3,4-C=N group of quinazoline (which is known to add the anions of weak acids [9]) and gave 3,4-dihydro-4-(7-hydroxypteridin-6-ylmethyl)quinazoline (III). The constitution of this adduct follows from the high basic strength (pK_a 9·26), which is similar to that of 3,4-dihydroquinazoline (9·19),[9a] and the presence of other pK values and ultraviolet spectra (see Table 1), corresponding to those of 7-hydroxypteridine (see p. 3360). That



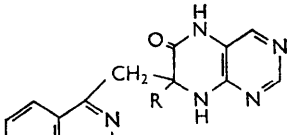
(I: R = Me) ('Substance T')
(II: R = H)



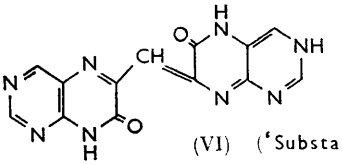
(III)

7-hydroxy-6-methylpteridine is not a Schiff–Michael receptor is shown from its surviving (quantitatively) boiling pH 2·5 buffer for 3 hours (lack of ability to undergo self-condensation).

That the 7,8-double bond of 6-hydroxypteridine is a carbanion receptor in acid solution was demonstrated above by the use of acetylacetone. However, it was desired to confirm this by using a closer analogue of 7-hydroxy-6-methylpteridine as the carbanion donor. 4-Methylquinazoline was chosen as the donor after it had been shown that the methyl group was active enough to combine with *m*-nitrobenzaldehyde, at pH 2·5, to give 4-3′-nitrostyrylquinazoline. We found that 4-methylquinazoline added to 6-hydroxypteridine and 6-hydroxy-7-methylpteridine, at pH 2·5, to give 7,8-dihydro-6-hydroxy-7-(quinazolin-4-ylmethyl)pteridine (IV), and its 7-methyl homologue (V), respectively. The excellent



(IV: R = H)
(V: R = Me)



(VI) ('Substance U')

yield of the adduct (V) was gratifying because the methyl-group in 6-hydroxy-7-methylpteridine exerts a partial steric hindrance to the addition of water across the 7,8-position.[3] That the 7-methyl group of the pteridine has not added across the 3,4-bond of the quinazoline (which is even more strongly sterically hindered by the 4-methyl group [9]) is shown by the similarity in spectra and pK values of (IV) and (V) (see Table 1).

As expected, 7-hydroxy-6-methylpteridine did not react appreciably with 4-methylquinazoline, under the above conditions. However 6-hydroxy-7-methylpteridine has both donor and receptor properties and hence, at pH 2·5, the methyl group can add across the 7,8-bond of another molecule of the same substance. This dimer, the principal source

* See p. 3360 for constitution.

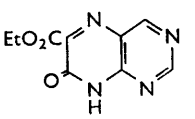
[4] Potts and Smith, *J.*, 1957, 4018.
[5] Ingold, " Structure and Mechanism in Organic Chemistry," Bell, London, 1953, p. 693.
[6] Schiff, *Ber.*, 1898, **31**, 205, 601.
[7] Lazzareschi, *Gazzetta*, 1937, **67**, 371.
[8] Albert and Howell, *J.*, 1962, 1591.
[9] Albert, Armarego, and Spinner, *J.*, 1961, (*a*) 2689; (*b*) 5267.

of loss in preparing " Substance T," readily loses two hydrogen atoms in air to give an orange solid, " Substance S."

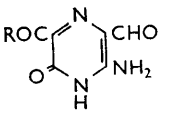
When " Substance T " was oxidized with aqueous alkaline potassium permanganate, the principal product was 6,7-dihydroxypteridine. Although this degradation is in agreement with formula (I), a more satisfying variant was provided by working with the lower homologue (II), readily prepared from 7-hydroxy-6-methylpteridine and 6-hydroxypteridine at pH 2·0. This homologue is readily oxidized by potassium ferricyanide, with loss of two hydrogen atoms, to the intensely orange-coloured " Substance U " (VI), *viz.*, 3,7-dihydro-6-hydroxy-7-(7-hydroxypteridin-6-ylmethylene)pteridine. This is a feebly basic substance, with a long conjugated pathway. As a substrate for oxidative degradation, it has the advantage over " Substance T " that it cannot undergo alkaline retrogression to the hydroxymethylpteridines and hence the products of oxidation cannot arise from the latter.

When " Substance U " was oxidized with cold, alkaline potassium permanganate, 7-hydroxypteridine-6-carboxylic acid and 6,7-dihydroxypteridine were obtained. This result not only justifies the disposition of the methylene double bond in formula (VI) but also confirms the constitution of substance (II) and hence of (I) (" Substance T "). The dihydroxypteridine was identical in physical properties with a standard specimen [10] of 6,7-dihydroxypteridine; the acid, hitherto unknown, was identical with an authentic specimen prepared from 4,5-diaminopyrimidine (as below), and was decarboxylated to 7-hydroxypteridine, a substance of markedly different properties [10] from 6-hydroxypteridine.

Ethyl 7-hydroxypteridine-6-carboxylate (VII) was made in good yield by the action of diethyl oxomalonate on 4,5-diaminopyrimidine in cold, faintly alkaline solution. Cold N-sodium hydroxide hydrolysed it to the free acid. The ester, in boiling pH 3 buffer, gave ethyl 2-amino-3-formyl-6-hydroxypyrazine-5-carboxylate (VIII; R = OEt), which was hydrolysed by cold N-sodium hydroxide to the corresponding acid.



(VII)



(VIII)

The low melting point (140°) of the pteridine ester (VII) and its high solubility in benzene, in contrast to the properties of 7-hydroxypteridine, suggested that internal hydrogen bonding may involve the carbonyl oxygen atom of the ester group with a true hydroxy-group in position 7. However, the infrared spectrum (potassium bromide disc) discounted this suggestion. Two strong carbonyl-stretching bands were found at 1740 (ester) and 1694 cm.$^{-1}$ (lactam). The 4-methyl derivative of the ester (VII) was made by condensing diethyl oxomalonate with 4,5-diamino-6-methylpyrimidine, and both esters were examined by rapid reaction methods [11] for covalent hydration; but this was not found, in spite of the ease of opening of what is apparently the less polarized ring.

Consonant with a generalization [12] that 6-hydroxypteridines can seldom be made from oxomalonic acid and esters, we could not obtain ethyl 6-hydoxypteridine-7-carboxylate from diethyl oxomalonate and 4,5-diaminopyrimidine in N-sulphuric acid. Pfleiderer [13] was able to obtain derivatives of 6-hydroxypteridine-7-carboxymethylamide by the action of 1,3-dimethylalloxan (a source of oxomalonic acid monomethylamide) on 4,5-diaminopyrimidines in mildly acidic solution. However, we found that 4,5-diaminopyrimidine and dimethylalloxan gave, at pH 6, exclusively 7-hydroxypteridine 6-carboxymethylamide, whereas at pH 2 no reaction took place, even when the dimethylalloxan was pretreated with one equivalent of sodium hydroxide. This methylamide, when

[10] (*a*) Albert, Brown, and Cheeseman, *J.*, 1952, 1620; (*b*) Albert, *J.*, 1955, 2690.
[11] Perrin, *J.*, 1960, 3189; 1962, 645.
[12] Pfleiderer, *Chem. Ber.*, 1957, **90**, 2624.
[13] Pfleiderer, *Chem. Ber.*, 1955, **88**, 1625.

boiled with water, gave 2-amino-3-formyl-6-hydroxypyrazine-5-carboxymethylamide (VIII; R = NHMe), which was also formed by the action of methylamine on the ester (VII), thus establishing the orientation of the hydroxy-group. All three pyrazine amino-aldehydes are highly stable.

Ionization Constants and Spectra.—These physical properties (see Table 1) add powerful support to the conclusions reached regarding the structure of " Substance T " and the other dipteridinylmethanes. First, the close agreement in pK values and ultraviolet spectra, between substances (I) and (II), and between (IV) and (V), shows that these form two pairs of homologues, and that the presence of the methyl group in the receptor molecule (6-hydroxy-7-methylpteridine) has not sterically prevented the addition of the donor molecule to the 7,8-bond.

The pK_a values of substances (I) and (II) reflect those of 7-hydroxy-6-methylpteridine (6·97 acidic, and 1·20 * basic) and of 7,8-dihydro-6-hydroxy-7-methylpteridine (10·89 * acidic, and 4·80 basic) (see ref. 3 and Table 1). This is what would be expected from the hypothesis outlined above.

The $\lambda_{max.}$ values of the ultraviolet spectra of the neutral species of substances (I) and (II) correspond to the long wave-band in both 7-hydroxy-6-methylpteridine [299 mμ (log ε 4·09)] and 7,8-dihydro-6-hydroxy-7-methylpteridine [294 mμ (log ε 3·95)] but the extinction coefficients are almost twice as great, as would be expected from the coincidence of $\lambda_{max.}$ values in the constituent parts. The cationic spectra of compounds (I) and (II) are similarly related to those of their constituents. The lack of recorded absorption in the 200—230 mμ region of compounds (I) and (II) is due to general absorption, but the 257 mμ peak of 7-hydroxy-6-methylpteridine is suppressed.

The pK_a values of compounds (IV) and (V) reflect those of 7,8-dihydro-6-hydroxy-7-methylpteridine (see above). Their ultraviolet spectra also closely follow those of 7,8-dihydro-6-hydroxy-7-methylpteridine (see above) for the longer wavelength peak (where 4-methylquinazoline absorbs relatively weakly) and of 4-methylquinazoline [9a] for the shorter wavelength peak (where this substance absorbs more intensely than the pteridine). 7-Diacetylmethyl-7,8-dihydro-6-hydroxypteridine (see Table 1) also resembles the other 7,8-dihydro-6-hydroxypteridines [3] in the cationic pK_a, and the spectra of cation and neutral species. Attention has already been drawn to the constitutionally diagnostic value of the high basic strength of compound (III) (see p. 3358).

The absorption at long wavelength of " Substance U " indicates a long conjugated pathway and justifies the placing of the mobile hydrogen atom at N-3 in formula (VI). " Substance U " is related to the pterorhodins,[14] and is in fact isopterorhodin [14a] stripped of two amino- and two hydroxy-groups. The pK values and spectra of the three pyrazine aldehydes are consistent with those of other 2-aminopyrazine-3-aldehydes.[1,15]

The mechanism of these acid-catalysed Schiff–Michael reactions is likely to resemble that of the usual base-catalysed Michael reaction in which a carbanion (on the donor) attacks a fractionally positively charged carbon atom (on the receptor). In the acid-catalysed reaction the donor need not be protonated, *e.g.*, acetylacetone could not be appreciably protonated at pH 2·5 where it is highly susceptible to this reaction. If, however, the donor is protonated, ionization of an α- or γ-methyl group is greatly facilitated, and the donor becomes a zwitterion stabilized by resonance with a methide of the type 1,2-dihydro-2-methylenepyridine.

Protonation of the receptor should be advantageous because it induces an increased positive charge on the carbon atom of the receptive HC=N group.

Optical Resolution on Paper (D-Cellulose).—Whereas compounds (I) and (II) give the

* A little coulombic weakening of these ionizations inevitably occurs in substances (I) and (II), where they are second ionizations.

[14] (*a*) Purrmann and Maas, *Annalen*, 1944, **556**, 186; (*b*) Russell, Purrmann, Schmitt, and Hitchings, *J. Amer. Chem. Soc.*, 1949, **71**, 3412.

[15] Perrin, *J.*, 1962, 645.

TABLE 1.

Physical properties of some pteridines and pyrazines.

Compounds	Species *	Ionization (H_2O; 20°) pK_a	Concn. (M)	A.w.l.§	Light absorption in water ¶ $\lambda_{max.}$ (mμ)	log ε	pH
Pteridines							
(I)	++	0·48 ± 0·04	10^{-4}	325	—	—	—
	+	4·47 ± 0·03	0·01	—	294	4·30	2·0
	0	—	—	—	298	4·23	5·63
	—	6·79 ± 0·03	0·01	—	305	4·11	9·1
	−−	11·07 ± 0·04	0·01	—	313	4·25	13·0
(II)	++	0·73 ± 0·04	10^{-4}	280	—	—	—
	+	4·50 ± 0·02	0·005	—	297	4·30	2·6
	0	—	—	—	298	4·25	5·7
	—	6·85 ± 0·04	0·005	—	*264*, 305	*3·92*, 4·12	9·0
	−−	11·03 ± 0·05	10^{-4}	320	—	—	—
7-Diacetylmethyl-7,8-dihydro-6-hydroxypteridine	+	4·45 ± 0·01	0·005	—	210, 292	4·41, 4·06	2·0
	0	—	—	—	209, 292	4·49, 4·05	6·0
	—	7·57 ± 0·05 †	0·005	—	209, 296	4·54, 4·43	9·5
(III)	++	0·83 ± 0·06	10^{-5}	320	*216*, 291	*4·54*, 4·20	−1·4
	+	6·42 ± 0·05	10^{-5}	229	*216*, 299	*4·46*, 4·12	3·0
	±	—	—	—	214, 264, 326	4·55, 3·90, 4·05	7·85
	—	9·26 ± 0·05	10^{-5}	229	214, *229*, 265, 327	4·54, *4·34*, 3·89, 4·06	11·5
(IV)	+	4·29 ± 0·04	10^{-4}	294	224, 295	4·56, 4·09	2·0
	0	—	—	—	224, 293	4·53, 3·96	7·0
	—	10·60 ± 0·04	10^{-4}	310	304	4·04	13·0
(V)	+	4·26 ± 0·05	10^{-4}	300	225, 297	4·56, 4·07	2·0
	0	—	—	—	224, 292	4·50, 3·91	7·8
	—	11·00 ± 0·05	10^{-4}	310	220, 305	4·64, 4·05	13·0
(VI)	0	‡ —	—	—	213, 319, 433, 457	4·58, 4·09, 4·47, 4·44	4·5
	—	7·01 ± 0·04	10^{-5}	475	219, 330, 426, 453	4·50, 4·05, 4·48, 4·46	8·1
	−−	9·23 ± 0·04	10^{-5}	475	222, 333 + 343, 428, 452	4·56, 4·09, 4·41, 4·48	11·5
(VII)	0	—	—	—	217, 251—259,‖ 321	4·24, 3·54, 3·97	3·3
	—	5·53 ± 0·02	10^{-4}	300	231, 259—265, 343	4·32, 3·78, 3·97	7·7
6-Ethoxycarbonyl-7-hydroxy-4-methylpteridine	0	—	—	—	221, 256—268, 321	4·22, 3·43, 3·98	3·5
	—	5·74 ± 0·04	10^{-4}	300	234, *264*, 340	4·34, *3·73*, 3·96	8·0
6-Carboxy-7-hydroxy-pteridine	+	—	—	—	220, 271—278, 319	4·31, 3·71, 3·87	−1·1
	±	1·50 ± 0·05	10^{-4}	300	<215, 260—264, 307	>4·25, 3·54, 4·06	4·0
	—	6·73 ± 0·03	10^{-4}	350	213, 254—259, 331	4·33, 3·72, 4·08	9·0
7-Hydroxy-6-methylcarbonamidopteridine	0	—	—	—	<215, 259—266, 323	>4·25, 3·63, 4·04	3·2
	—	5·70 ± 0·02	0·005	—	230, *260*, 349	4·36, *3·86*, 4·00	8·2
7-Hydroxy-6-methylpteridine	+ **	1·20 ± 0·04	10^{-4}	270	294	4·14	−1·0 ††
	0	—	—	—	257, 299	3·54, 4·09	4·7
	—	6·97 ± 0·03	0·02	—	261, 324	3·66, 4·12	9·2
Pyrazines							
(VIII; R = OEt)	0	—	—	—	230, 293, 361	3·89, 4·14, 4·40	3·5
	—	6·09 ± 0·03	10^{-4}	230	229, 293, 360	4·30, 4·14, 4·34	8·5
(VIII; R = OH)	0	—	—	—	230, 291, 363	3·85, 4·12, 4·38	1·2
	—	3·71 ± 0·02	10^{-4}	315	223, 290, 358	4·19, 4·07, 4·26	5·7
	−−	7·63 ± 0·03	10^{-4}	256	220, 256, 295, 350	4·13, 3·81, 4·00, 4·27	10·1
(VIII; R = NHMe)	0	—	—	—	229, 295, 360	3·98, 4·11, 4·39	3·5
	—	6·01 ± 0·03	10^{-4}	230	229, 295, 359	4·36, 4·12, 4·36	8·5

* Dication (++), cation (+), neutral species (0), anion (−), dianion (−−) and zwitterion (±). † Ionization in the side-chain (*cf.* acetylacetone, pK 8·2); the dianion is >11·5. ‡ Cation is <1·7. § Analytical wavelength in mμ; an entry in this column indicates a spectrometric (otherwise potentiometric) determination (ref. 17). ¶ Inflections in italics. ‖ Flat area. ** No figures for this species have been recorded previously; data for the anion and neutral species are from ref. 3. †† H_0 value in hydrochloric acid.

twin spots on a paper chromatogram, (IV)—(VI) do not. Recently, a complex pteridine, (±)-L-tetrahydro-5,10-methanofolate anion, has been resolved on a triethylaminoethyl–cellulose ion-exchange column.[16] Many racemic amino-acids, usually having an aromatic ring, give twin spots on paper chromatograms and the R_F of one spot (in each case) has been shown to correspond to the natural L-acid, although no spots have been eluted or rotations measured (see ref. 2 for a summary).

Experimental

Microanalyses were by Dr. J. E. Fildes, ionization constants by Dr. D. D. Perrin (using methods developed in this Department [17]), and spectra by Dr. E. Spinner, and their respective staffs. Solids for analysis were dried at 110° in air, unless otherwise stated. Apart from substances (I) and (II), yields refer to material sufficiently pure to give only one spot on paper chromatography (see Table 2 for conditions). The infrared spectra were observed for KBr discs.

Table 2.

Data from paper * chromatography (ascending method).

	R_F and colour of spot in ultraviolet light					
	In butanol–5N-acetic acid (7 : 3) (pH 2·7)			In 3% ammonium chloride (pH 5·5)		
Substance †	R_F	254 mμ	365 mμ	R_F	254 mμ	365 mμ
4,5-Diaminopyrimidine	0·30	D ¶	X	0·65	O	X
7-Hydroxy-6-methylpteridine	0·65 ‡	D → B ‖	X	0·70	D → B	X
6-Hydroxy-7-methylpteridine	0·60 ‡	V	V	0·70	D	D
7-Hydroxypteridine	0·50	D → B ‖	X	0·65	D → B	X
6-Hydroxypteridine	0·4—0·8	D	X	0·05	D	X
6,7-Dihydroxypteridine	0·25	B	B **	0·55	B	B **
(I)	0·35 §	D → B ‖	X	0·65 0·55	D → B	X
(II)	0·20	D	X	0·50 0·40	D	Y
Quinazoline	0·90	D	X	0·0	D	X
(III)	0·50	W	W	0·60	W	Y
(IV)	0·3—0·6	Y	Y	0·45	Y	Y
(V)	0·75	D	X	0·60	D	X
(VI)	0·0—0·2	Y	Y	0·0	Y	Y
7-Diacetylmethyl-7,8-dihydro-6-hydroxypteridine	0·45	D	B	0·75	D	X
6-Ethoxycarbonyl-7-hydroxypteridine (VII)	0·80	D → B ‖	X	0·75	D → B	X
6-Carboxy-7-hydroxypteridine	0·20	D	X	0·80	D	X
7-Hydroxy-6-methylcarbonamidopteridine	0·45	D → B ‖	X	0·65	D → B	X
Pyrazines (VIII)						
R = OEt	0·65	2B **	3V	0·65	2B **	3V
R = OH	0·25	2B **	3V	0·50	2B **	3V
R = NHMe	0·50	2B **	3V	0·55	2B **	3V

* Whatman's No. 1. † Dissolved in pyridine trihydrate or 0·1N-sodium hydroxide. ‡ These isomers are distinguished by development in 0·1N-hydrochloric acid (2 days at 0°), when 7-hydroxy-6-methylpteridine has R_F 0·75 D → B; X, and 6-hydroxy-7-methylpteridine has R_F 0·85 B; B. § Optical resolution can also be brought about by development for 2 days at 0° in a mixture of propan-2-ol (65), dimethylformamide (22·5), formic acid (2·5), and water (10 ml.). ¶ D, dark (*i.e.*, absorbing, non-fluorescent); X, does not show; B, V, W, Y, blue, violet, white, yellow fluorescences, respectively. ‖ The change from D to B is caused by the lamp, and is believed to be a photo-reduction of the 7,8-bond. ** Much fainter under this, than under the other lamp.

"Pyridine trihydrate" refers to the constant-boiling (93°) mixture of pyridine and water.

"*Substance T*" (I).—(*a*) 4,5-Diaminopyrimidine [18] (3·3 g., 0·03 mole) was dissolved in N-sulphuric acid (60 ml.), and N-sodium hydroxide (about 27 ml.) added to give pH 2·5. The

[16] Kaufman, Donaldson, and Keresztesy, *J. Biol. Chem.*, 1963, **238**, 1498.
[17] Albert and Serjeant, "Ionization Constants," Methuen, London, 1962; Perrin, *Austral. J. Chem.*, 1963, **16**, 572.
[18] Brown, *J. Appl. Chem.*, 1952, **2**, 239.

solution was refluxed with ethyl pyruvate [19] (3·6 g., freshly distilled) for 3 hr., cooled, adjusted to pH 3·3 with M-sodium citrate and 10N-sodium hydroxide, and filtered at once from a copious orange precipitate of " Substance S " (2 g., see below). The filtrate, adjusted to pH 5·6 with 10N-sodium hydroxide and refrigerated overnight, gave a precipitate (2 g.) which was refluxed with boiling water for 20 min. and filtered as hot as possible. The filtrate, concentrated to 25 ml. at 40°, gave 7,8-*dihydro*-6-*hydroxy*-7-(7-*hydroxypteridin*-6-*ylmethyl*)-7-*methylpteridine* (I) (20%). Heat is essential for this reaction, and the yield is higher after 3 hr. than after 1 or 12 hr. A trace of yellow impurity was removed by chromatography in 2N-ammonia on alumina.

" Substance T " was an ivory-white solid (from water), unchanged on heating in air at 170°, but decomposing rapidly at 280° (Found, for material dried at 110°/0·01 mm.: C, 49·4; H, 4·1; N, 32·9. $C_{14}H_{12}N_8O_2,H_2O$ requires C, 49·1; H, 4·1; N, 32·7%. Found, for material dried at 140°: C, 52·0; H, 3·7; N, 34·8. $C_{14}H_{12}N_8O_2$ requires C, 51·85; H, 3·7; N, 34·6%), $\nu_{max.}$ (for material dried at 110°) 3405 (H_2O of crystallization), 3215, 3040 (NH stretching), 1686 (C=O stretching), 1620, 1595, 1547, 1469, 1412 cm.$^{-1}$. Potentiometric titration showed that the molecular weight is 324, or a multiple of this. The relatively high solubility in boiling water (1 in 350) indicates that the value does not exceed 324 (cf. 6,7-dihydroxypteridine, 1 in 290, and 2,4,6,7-tetrahydroxypteridine, 1 in 7000). Measurement of freezing-point depression of a 0·1M-solution in 0·1M-hydrochloric acid, using a thermistor in a Wheatstone bridge circuit calibrated with quinazoline, confirmed that the molecular weight of " Substance T " is approximately double that of quinazoline (Found $-\Delta T$ 0·290°, expected ~0·33°). " Substance T " was unaffected by boiling N-hydrochloric acid, but when it (0·2 g.) was refluxed with 0·5N-sodium hydroxide (4 ml.) for 1 hr., retrogression of the original condensation gave 7-hydroxy-6-methylpteridine (0·08 g.) (precipitated at pH 2·5) and 6-hydroxy-7-methylpteridine (0·06 g.) (precipitated from this filtrate, at pH 5·5). " Substance T " was destroyed by ultraviolet light, even at 365 mμ.

(*b*) (Recommended method). 7-Hydroxy-6-methyl- [3] and 6-hydroxy-7-methyl-pteridine [3] (0·3 g. of each), N-sulphuric acid (6 ml.), and 0·5N-sodium hydroxide (6 ml.) were refluxed for 3 hr. (the optimal time). The pH of the mixture, which had remained at 2·5, was adjusted to 5·6. The crystals (two crops) which separated slowly during refrigeration were refluxed with water for 20 min., and filtered as hot as possible. The filtrate gave " Substance T " (40%) on concentration.

Optical Resolution of Racemate.—" Substance T " (50 mg.) in 0·01M-borate buffer (25 ml.; pH 8·8) was applied to a column of cellulose (4·7 × 120 cm.), previously equilibrated at 1° with the buffer, flowing at about 60 ml./hr. The column was developed with the same buffer at this temperature. Ultraviolet spectroscopy of the eluate revealed two fractions which were concentrated separately at 40° and adjusted to pH 5·6. The material deposited during refrigeration was recrystallized from water, as above, and the rotation measured with a polarimeter (Bellingham and Stanley's model A). The specific rotations are given in the Introduction; that of the faster-running fraction was positive. An attempt to use the same column of cellulose to resolve a further portion of " Substance T " was unsuccessful, but a fresh column behaved as before.

7,8-*Dihydro*-6-*hydroxy*-7-(7-*hydroxypteridin*-6-*ylmethyl*)*pteridine* (II).—7-Hydroxy-6-methylpteridine (0·96 g., 0·006 mole), 6-hydroxypteridine hydrate [10b] (1·02 g., 0·002 mole), N-sulphuric acid (18 ml.), water (75 ml.), and enough N-sodium hydroxide to give pH 2·0 (about 4·5 ml.) were refluxed under nitrogen for 3 hr. and filtered. The filtrate, adjusted to pH 5·6, gave a precipitate which was collected, boiled with water (30 ml.), and filtered. The *dipteridinylmethane* (II) (70%) was an ivory solid, unchanged at 200°, and very sparingly soluble in water and common organic solvents (Found: C, 49·8; H, 3·5; N, 35·8. $C_{13}H_{10}N_8O_2$ requires C, 50·3; H, 3·3; N, 36·1%). Although it became superficially orange when exposed to the air (formation of " Substance U," see below), the rate of aerial oxidation in boiling N-sodium hydroxide or N-sulphuric acid was too slow for preparative purposes, even in the presence of finely divided platinum.

7-*Diacetylmethyl*-7,8-*dihydro*-6-*hydroxypteridine*.—6-Hydroxypteridine hydrate [10b] (0·17 g., 0·001 mole), acetylacetone (0·5 ml., 5 equiv.), N-sulphuric acid (2 ml.), and enough N-sodium hydroxide (about 1·2 ml.) to give pH 2·5 were refluxed for 1 hr. (final pH 2·3). The clear pale brown solution, adjusted to pH 6 and cooled, gave the *diacetylmethylpteridine* (90%),

[19] Archer and Pratt, *J. Amer. Chem. Soc.*, 1944, **66**, 1656; Howard and Fraser, *Org. Synth.*, Coll. Vol. I, 1948, p. 475.

which began to darken at 270° (from ethanol) (Found: C, 53·4; H, 5·2; N, 22·6. $C_{11}H_{12}N_4O_3$ requires C, 53·2; H, 4·9; N, 22·6%).

3,4-*Dihydro*-4-(7-*hydroxypteridin*-6-*ylmethyl*)*quinazoline* (III).—7-Hydroxy-6-methylpteridine [3] (0·16 g., 0·001 mole), quinazoline [20] (0·13 g., 0·001 mole), N-sulphuric acid (2 ml.), and enough N-sodium hydroxide (about 1 ml.) to give pH 2·5, were boiled until dissolved (2 min.), and set aside at 25° for 2 days. The mixture was adjusted to pH 7 and filtered. The cake, washed with ethanol and dried at 20°, gave the *quinazoline* (85%), m. p. 216° (from water), soluble in pyridine trihydrate (Found: C, 61·4; H, 4·6; N, 28·6. $C_{15}H_{12}N_6O$ requires C, 61·6; H, 4·15; N, 28·8%).

4-3′-*Nitrostyrylquinazoline*.—4-Methylquinazoline [21] (0·14 g., 0·001 mole), *m*-nitrobenzaldehyde (0·18 g.), N-sulphuric acid (2 ml.) and enough N-sodium hydroxide to give pH 2·5 were refluxed for 2 hr., cooled, and adjusted to pH 4 with M-sodium citrate. The solid was filtered off and triturated with alcohol, to give 4-3′-*nitrostyrylquinazoline* (55%), m. p. 188° (from pyridine trihydrate) (Found: C, 68·9; H, 4·2; N, 15·1. $C_{16}H_{11}N_3O_2$ requires C, 69·3; H, 4·0; N, 15·2%).

Adducts of 4-*Methylquinazoline*.—6-Hydroxypteridine hydrate [10b] (0·17 g., 0·001 mole), 4-methylquinazoline [21] (0·14 g., 0·001 mole), N-sulphuric acid (2 ml.), and enough N-sodium hydroxide (about 1·2 ml.) to give pH 2·5 at 20° were refluxed under nitrogen for 1 hr., cooled, and adjusted to pH 7. The precipitate was dissolved in 0·5N-sodium hydroxide (3 ml.) and filtered; the filtrate, adjusted to pH 7, deposited 7,8-*dihydro*-6-*hydroxy*-7-(*quinazolin*-4-*ylmethyl*)*pteridine* (IV), (80%). It began to char at 280° and was very slightly soluble in boiling water and organic solvents. The pale yellow solution in N-sodium hydroxide became scarlet when aerated (Found, for material dried at 20°/0·01 mm.: C, 61·1; H, 4·0; N, 29·0. $C_{15}H_{12}N_6O$ requires C, 61·6; H, 4·15; N, 28·8%).

6-Hydroxy-7-methylpteridine hydrate [3] (0·18 g., 0·001 mole), 4-methylquinazoline (0·14 g.), N-sulphuric acid (2 ml.), and enough N-sodium hydroxide to give pH 2·5 were set aside at 28° for 10 days. The small amount of precipitate was filtered off and discarded. The filtrate, adjusted to pH 7, gave a colourless precipitate of 7,8-*dihydro*-6-*hydroxy*-7-(*quinazolin*-4-*ylmethyl*)-7-*methylpteridine* (V) (60%), turning brown at about 240° (Found, for material from pyridine trihydrate dried at 110°/0·01 mm.: C, 62·4; H, 4·7; N, 27·4. $C_{16}H_{14}N_6O$ requires C, 62·7; H, 4·6; N, 27·4%). Boiling water splits it to the original pteridine and quinazoline.

" *Substance S* " [*Presumed* 3,7-*Dihydro*-6-*hydroxy*-7-(7,8-*dihydro*-6-*hydroxy*-7-*methylpteridin*-7-*ylmethylene*)*pteridine*.—6-Hydroxy-7-methylpteridine (0·2 g.), N-sulphuric acid (2 ml.), and enough N-sodium hydroxide to give pH 2·5 were refluxed for 1 hr. The orange suspension was adjusted to pH 5 and filtered. The insoluble residue was ground under water (15 ml.), boiled for 20 min., and filtered as hot as possible. The residue was rapidly dissolved in 0·1N-sodium hydroxide (in which it was unstable), filtered, and the filtrate adjusted to pH 5. The hygroscopic orange precipitate of " *Substance S* " was insoluble in all common solvents, and unchanged at 300° (Found, for material dried at 110°/0·01 mm.: C, 50·8; H, 4·0; N, 33·2; loss on drying at 150°: 1·3. $C_{14}H_{12}N_8O_2,\frac{1}{3}H_2O$ requires C, 50·9; H, 3·9; N, 33·9; H_2O, 1·8%), $\lambda_{max.}$ (0·1N-sodium hydroxide) 298, 433, *540* mμ (log ε 4·08, 3·71, *3·11*).

3,7-*Dihydro*-6-*hydroxy*-7-(7-*hydroxypteridin*-6-*ylmethylene*)*pteridine* (" *Substance U* ") (VI).—To the dipteridinylmethane (II) (see above) (0·47 g., 0·0015 mole) in N-potassium hydroxide (7·5 ml.) was added potassium ferricyanide (1·0 g.; 1 equiv.) in water (5 ml.). The solution, after 40 hr. at 25°, was acidified to pH 2·5, and filtered. The cake was boiled for 5 min. with water (15 ml.) and filtered as hot as possible. The insoluble " Substance U " (65%) was dissolved in N-sodium hydroxide (10 vol.) from which alcohol (5 vol.) precipitated the orange sodium salt. " Substance U " was regenerated in water at pH 2·5, boiled with water (15 ml., discarded), and dried at 150° (Found: C, 50·3; H, 2·9; N, 35·3. $C_{13}H_8N_8O_2$ requires C, 50·6; H, 2·6; N, 36·4%). It is orange, sparingly soluble in N-mineral acids, virtually insoluble in all common organic solvents, and unchanged at 300°.

Oxidation of " *Substance U*."—0·1M-Potassium permanganate (20 ml.; 6 H equiv.) was added, during 20 min., to a well-shaken solution of " Substance U " (0·31 g., 0·001 mole) in N-potassium hydroxide (1·5 ml.) at 25°. Kieselguhr (0·5 g.) was added, and the mixture filtered. The filtrate, acidified to pH 2 with 5N-sulphuric acid, gave crystals on refrigeration. These, dissolved in N-potassium carbonate (3 ml.), deposited the potassium salt of 6,7-dihydroxypteridine,

[20] Armarego, *J. Appl. Chem.*, 1961, **11**, 70.
[21] Siegle and Christensen, *J. Amer. Chem. Soc.*, 1951, **73**, 5777.

which, in water at pH 5, gave 6,7-dihydroxypteridine [10] (0·15 g.); the filtrate, adjusted to pH 2, deposited 7-hydroxypteridine-6-carboxylic acid (0·07 g.) (see below).

Ethyl 7-Hydroxypteridine-6-carboxylate (VII).—4,5-Diaminopyrimidine [18] (1·1 g., 0·01 mole), N-potassium carbonate (15 ml.), diethyl oxomalonate (2·6 g., 1·5 equiv.), and water (7 ml.) were set aside at 20° for 44 hr. (pH is 8), then adjusted to pH 4·5 with 5N-sulphuric acid. The *ester* (80%) had m. p. 140° (from benzene) (Found: C, 49·2; H, 3·5; N, 25·5. $C_9H_8N_4O_3$ requires C, 49·1; H, 3·7; N, 25·4%), soluble in 1·5 parts of boiling alcohol. This ester (0·88 g.) in N-sodium hydroxide (8 ml., 2 equiv.) was set aside at 20° for 20 min., then adjusted to pH 2 with 5N-sulphuric acid. The precipitate of *7-hydroxypteridine-6-carboxylic acid* was collected, ground under water, filtered off, and dried at 65°/0·001 mm. No suitable solvent for recrystallization was found. It turned violet on heating to 100° (Found: C, 43·4; H, 2·15; N, 28·9. $C_7H_4N_4O_3$ requires C, 43·8; H, 2·1; N, 29·2%).

Ethyl 2-Amino-3-formyl-6-hydroxypyrazine-5-carboxylate (VIII; R = OEt).—The above pteridine ester (1 g.) and citrate buffer (20 ml.; pH 3·0) were refluxed for 20 min., and adjusted to pH 4·7. The precipitate, after extraction with boiling benzene gave the *pyrazine ester* (20%), darkening at 250° without melting (from water then pyridine) (Found: C, 45·5; H, 4·3; N, 20·0. $C_8H_9N_3O_4$ requires C, 45·5; H, 4·3; N, 19·9%), $\nu_{max.}$ 3300, 3200, 2950, 2800 (NH stretching), 1720, 1670, 1630 (C=O stretching), and 1100 cm.$^{-1}$ (C–O stretching). This ester (0·36 g.) was set aside for one day at 20° in N-sodium hydroxide (4·5 ml.). The flask was warmed to dissolve the sodium salt, and the contents adjusted to pH 2 with 5N-sulphuric acid. The *acid* (VIII; R = OH) (90%) charred slightly at 260° without decarboxylating (from water) (Found: C, 39·4; H, 2·9; N, 22·9. $C_6H_5N_3O_4$ requires C, 39·4; H, 2·8; N, 22·95%), $\nu_{max.}$ 3350, 3250, 3050, 2700 (NH stretching), and 1735, 1660, 1610 cm.$^{-1}$ (C=O stretching). Like its ester (above) and methylamide (below), it gave a red precipitate with *p*-nitrophenylhydrazine at pH 2. This acid (0·66 g.) and pyridine (13 ml.) were refluxed for an hour. The solvent was evaporated and the residue, recrystallized from N-sodium hydroxide (9 ml.) as the sodium salt and regenerated in water at pH 4·5, gave 7-hydroxypteridine [10a] (65%).

Ethyl 7-Hydroxy-4-methylpteridine-6-carboxylate.—4,5-Diamino-6-methylpyrimidine [22] (0·62 g., 0·005 mole), N-potassium carbonate (8 ml.), and diethyl oxomalonate (1·3 g.), set aside at 22° for 52 hr. then adjusted to pH 4·5, gave the *ester* (80%), m. p. 211° (from ethanol), soluble in boiling water (Found: C, 51·5; H, 4·4; N, 23·7. $C_{10}H_{10}N_4O_3$ requires C, 51·3; H, 4·3; N, 23·9%).

7-Hydroxypteridine-6-carboxymethylamide.—A solution of 1,3-dimethylalloxan dihydrate [23] (2 g., 1 equiv.) in water (3 ml.) was added to 4,5-diaminopyrimidine (1·1 g., 0·01 mole) in N-sulphuric acid (5 ml.) and water (10 ml.). After effervescence had subsided, the solution (pH 6) was set aside overnight, then adjusted to pH 4·5. The *carboxymethylamide* (65%) decomposed slowly about 250° (from ethanol) (Found: C, 47·1; H, 3·5; N, 33·7. $C_8H_7N_5O_2$ requires C, 46·8; H, 3·4; N, 34·1%). This amide (0·5 g.), refluxed with water (75 ml.) for 3 hr. and cooled, gave *2-amino-3-formyl-6-hydroxypyrazine-5-carboxymethylamide* (VIII; R = NHMe) (50%), which was recrystallized from 1500 parts of boiling water (Found: C, 42·9; H, 4·15; N, 28·7. $C_7H_8N_4O_3$ requires C, 42·9; H, 4·1; N, 28·6%), $\nu_{max.}$ 3350, 3200, 2850 (NH stretching), and 1690, 1635, 1620 cm.$^{-1}$ (C=O stretching). It is very soluble in cold 0·1N (but deposits a sodium salt from N)-sodium hydroxide. This amide was also formed (85%) by heating ethyl 7-hydroxypteridine-6-carboxylate (0·5 g.) and 33% ethanolic methylamine (4 ml.) in a sealed tube at 110° for 3 hr.

We thank Drs. D. J. Brown, D. D. Perrin, E. Spinner, and W. L. F. Armarego for helpful discussions, and Mr. K. Tratt for experimental assistance.

Department of Medical Chemistry, John Curtin School of Medical Research, Australian National University, Canberra, Australia.

[Present address (E. P. S.): Royal Military College, Duntroon, Australia.]

[*Received, January 17th,* 1964.]

[22] Albert, Brown, and Wood, *J.*, 1954, 3832.
[23] Fischer, *Ber.*, 1881, **14**, 1912.