Tetrahedron Letters,Vol.28,No.32,pp3667-3670,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

PHYSAROCHROME A, A PLASMODIAL PIGMENT FROM THE SLIME MOULD PHYSARUM POLYCEPHALUM (MYXOMYCETES)[1]

Bert Steffan, Monika Praemassing and Wolfgang Steglich*

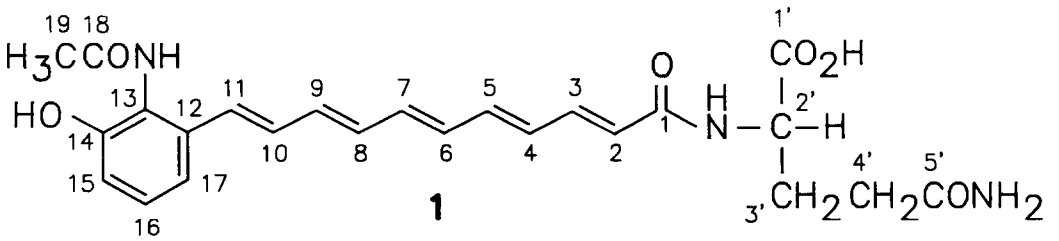
Institut für Organische Chemie und Biochemie der Universität Bonn
Gerhard-Domagk-Straße 1, D-5300 Bonn, West Germany

Abstract: From plasmodia of the slime mould Physarum polycephalum a yellow pigment physarochrome A (1) has been obtained. Its structure has been established as all-trans N-[11-(2-acetylamino-3-hydroxyphenyl)-2,4,6,8,10-undecapentaenoyl]-S-glutamine (1) on the basis of spectroscopic evidence and hydrogenation to a decahydro derivative.

The plasmodial pigments of Physarum polycephalum Schw. are considered to act as photoreceptors in this slime mould[2]. Despite several efforts to elucidate their structure the chemical nature of these pigments remained as yet unknown[3,4]. A recent publication on the separation of the pigments from P. polycephalum by means of HPLC[4] prompts us to disclose the structure of physarochrome A, one of the main pigments of this organism.

Physarochrome A can be isolated from the plasmodia[5] by extraction with methanol followed by chromatography on Sephadex LH 20 with methanol in the dark. The last of several yellow zones was purified by equilibration between ethyl acetate and citrate puffer at pH 4.5 and rechromatography of the pigment obtained from the organic phase on Sephadex LH 20. According to the ^{1}H NMR spectrum physarochrome A was contaminated with ca. 5% of a closely related pigment which could not be removed even by HPLC[6].

Physarochrome A forms a yellow amorphous powder, $[\alpha]_D$ = +7.2° (c 0.042 in MeOH), which exhibits absorption maxima (MeOH) at 213, 288 and 387 nm and IR bands (KBr) at 3320, 2920 (sh), 1720, 1660, 1600, 1460, 1380, 1365, 1350, 1285, 1270, 1190, 1120, 1060 and 1000 cm^{-1}. The compound gives a molecular ion m/z 453 in the FAB MS which is absent under electron impact conditions. The latter spectrum shows an ion m/z 417, $C_{24}H_{23}N_3O_4$, which corresponds to the loss of two molecules of water from the molecular ion $C_{24}H_{27}N_3O_6$.



1

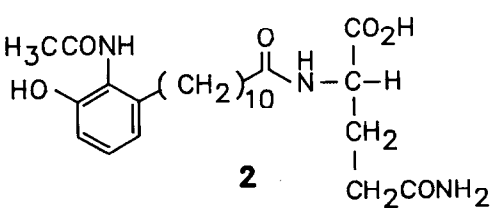
Table 1. ^{1}H NMR data of physarochrome A (**1**), δ in ppm and J in Hz. The spectrum was recorded at 400 MHz with [D_4]methanol (δ 3.35) as solvent and internal reference.

H	δ	mult.	J	H	δ	mult.	J
2	6.13	d	15.0	15	6.83	dd	7.6, 1.5
3	7.27	dd	15.0, 11.3	16	7.14	t	7.6
4,6	6.45	dd	14.6, 11.3	17	7.20	dd	7.6, 1.5
5	6.70	dd	14.6, 11.3	19	2.24	s	
7,8,9	6.56	m		2'	4.52	dd	8.0, 4.5
10	6.94	dd	15.1, 10.4	3a'	2.03	m	
11	6.72	d	15.1	3b'	2.25	m	
				4'	2.36	m	

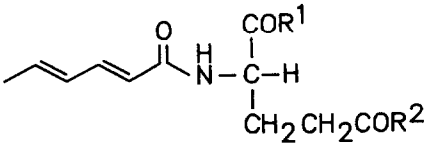
In the high field ^{1}H NMR spectrum (Table 1) signals for a decapentaene unit, a 1,2,3-trisubstituted benzene ring, an acetylamino group, and a glutamine residue are present. The assignment of the overlapping multiplets in the olefinic region was accomplished by COSY 2D-NMR experiments[7]. At 240 K in [D_6]acetone six exchangeable protons are visible, which can be attributed to the amide protons of the glutamine residue (δ 6.83, 7.38)[8], two secondary amide protons (δ 8.02, d, J = 8 Hz and 8.63, s), a phenolic hydroxyl (δ 9.35), and a carboxylic proton at 11.43 ppm.

The presence of the pentaene unit was confirmed by hydrogenation of physarochrome A with Pd on charcoal which yielded the colourless decahydro derivative **2**[9].

The ^{1}H-coupled ^{13}C NMR spectrum (Table 2) of physarochrome A is in complete agreement with structure **1**. Selective decoupling of 17-H leads to a simplification of the signals for C-11, C-13 (ddd → dd), and C-15 (Dd → D). This proves the attachment of the decapentaene chain to C-12 and the presence of the acetylamino group at C-13 (δ 136.83). The chemical shift of C-14 (δ 154.39) and its coupling both with 15-H (J = 2 Hz) and 16-H (J = 9 Hz) confirm the position of the phenolic OH group at C-14. Irradiation at the methyl frequency of the acetylamino group (δ 2.24) leads to collapse of the C-18 quartet and sharpening of the broadened ddd for C-13. The α-position of the amide group in the glutamine part was ascertained by comparison with the ^{13}C NMR data of N-(2,4-hexadienoyl)glutamine (**3**) and N-(2,4-hexadienoyl)isoglutamine (**4**), respectively[10]. As can be seen from Table 2, the signals for the carboxamide and carboxyl groups of **1** are virtually identical with those of **3**, however show distinct differences on comparison with the corresponding signals of **4**.



2



3: R^1 = OH, R^2 = NH_2

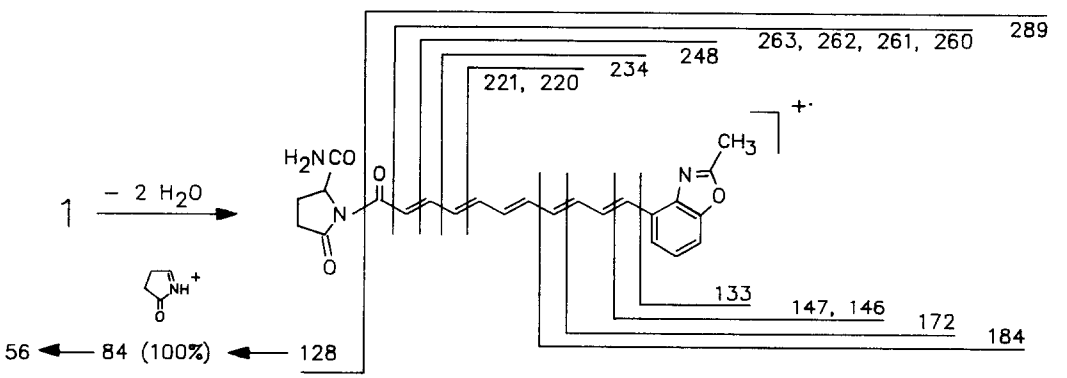
4: R^1 = NH_2, R^2 = OH

Table 2. ^{13}C NMR data of physarochrome A (**1**), N-(2,4-hexadienoyl)glutamine (**3**), and N-(2,4-hexadienoyl)isoglutamine (**4**), δ in ppm and J in Hz. The spectra were recorded at 100.62 MHz in [D_4]methanol (δ 49.0 ppm) as solvent and internal reference[a].

	1			**3**	**4**		**1**			**3**	**4**
	δ	mult.	J	δ	δ		δ	mult.	J	δ	δ
C-1	168.85	m		168.00	168.88	C-13	136.83	ddd	8,6,4		
C-2	123.95	Dm	158	122.24	122.35	C-14	154.39	dd	9,2		
C-3	142.40	Dm	152	142.27	142.74	C-15	116.55	Dd	160,8		
C-4	131.53	Dm	152	131.02	131.08	C-16	129.01	D	160		
C-6	133.75	Dm	155			C-17	117.60	Ddd	160,8,4		
C-7	134.74	Dm	156			C-18	173.36	q	6		
C-8	136.83	Dm	156			C-19	22.65	Q	128		
C-9	137.95	Dm	156			C-1'	175.13	m		175.05	176.30
C-5	141.27	Dm	152	139.13	139.14	C-2'	53.59	Dm	140	53.37	53.76
C-10	131.85	Dm	152			C-3'	28.81	Tm	128	28.62	28.56
C-11	130.28	Dm	152			C-4'	32.75	Tm	128	32.65	31.27
C-12	123.41	m				C-5'	177.76	m		177.69	176.30

[a] The signals were assigned by selective ^1H-^{13}C decouplings and 2D ^1H-^{13}C chemical shift correlation.

The mass spectral fragmentations of physarochrome A are depicted in Scheme 1[11]. The loss of two molecules of water from the molecular ion may be explained by formation of a benzoxazole ring from the o-acetaminophenol moiety and a pyroglutamyl ring from the glutaminyl residue. The latter transformation appearently occurs with migration of the amide nitrogen from C-5' to C-1' via a glutarimide intermediate. After release of the ketene fragment m/z 289 the resulting ion m/z 128 undergoes loss of a $CONH_2$ radical to yield the base peak at m/z 84. The formation of a prominent fragment m/z 84 is also observed in the MS spectrum of N-(2,4-hexadienoyl)-glutamine (**3**)[10], whereas the corresponding isoglutamine derivative[10] shows the same fragment only in 25.4% intensity.



Scheme 1: Mass spectroscopic fragmentations of physarochrome A (**1**)

The (S)-configuration of physarochrome A (**1**) was established by hydrolysis of its decahydro derivative **2** with 6 n HCl which yielded (S)-glutamic acid, whose configuration was proved by GC analysis of its N-pentafluoropropionyl diisopropylester on a Chirasil-D-Val column (Chrompack)[12].

Physarochrome A shows some structural similarities to fuligorubin A, a plasmodial pigment from the myxomycete Fuligo septica[13].

Acknowledgement - The work was supported by the Deutsche Forschungsgemeinschaft. We thank Prof. Wohlfahrt-Bottermann, Bonn, for his interest and continuous help in providing us with cultures of P. polycephalum. We are grateful to Dr. Ciommer, Hoechst AG, for the FAB MS spectra, to Dr. Eckhardt, Bonn, for the EI mass spectra and to Frau Hovenbitzer and Herrn Selbitschka, Bonn, for HPLC and GC separations.

REFERENCES AND NOTES

1. Pigments of Fungi, 55. - Part 54: E. Jaegers, E. Hillen-Maske, H. Schmidt, W. Steglich and E. Horak, Z. Naturforsch., in press.

2. L. Rakoczy in H. Senger (Ed.): The Blue Light Syndrome, p. 570, Springer, Berlin, Heidelberg, New York, 1980.

3. L. Rakoczy, Ber. Deutsch. Bot. Ges. **86**, 141 (1973).

4. A. Majcherczyk, L. Rakoczy and A. Hüttermann, Anal. Biochem. **160**, 178 (1987).

5. The plasmodia were cultured on oat flakes and harvested after 3 days.

6. Physarochrome A corresponds in its HPLC behaviour to peak 1 in figure 2 of ref. 4.

7. A. Bax and R. Freeman, J. Magn. Res. **44**, 542 (1981).

8. A. Bundi, Ch. Grathwohl, J. Hochmann, R. M. Keller, G. Wagner and K. Wüthrich, J. Magn. Res. **18**, 191 (1975).

9. **2**: $[\alpha]_D$ -21° (c 0.02 in MeOH); ^{1}H NMR ([D_4]methanol): δ 1.35 (br. m, 20H), 2.00 (m, 1H), 2.16 (m, 1H), 2.19 (s, 3H), 2.29 (m, 2H), 4.33 (dd, J = 8, 4.4 Hz, 1H), 6.75 (d, J = 7.8 Hz, 2H), 7.08 (t, J = 7.8 Hz, 1H); MS (70 eV, direct inlet 220°C, AEI-MS 50): m/z 427.2483 (M^+-2 H_2O, 12.9%, calc. for $C_{24}H_{33}N_3O_4$ 427.2495), 300 (13.7, $C_{19}H_{26}NO_2$), 258 (18.2, $C_{17}H_{24}NO$), 174 (15.7, $C_{11}H_{12}NO$), 161 (18.4, $C_{10}H_{11}NO$), 160 (80.2, $C_{10}H_{10}NO$), 148 (11.8, $C_9H_{10}NO$), 147 (100, C_9H_9NO), 146 (69.7, C_9H_8NO), 84 (18.9, C_4H_8NO).

10. **3**: m.p. 120°C; MS (180°C): m/z 222 (M^+-H_2O, 8.8%), 207 (15.3, $C_{10}H_{11}N_2O_3$), 95 (100, C_6H_7O), 94 (10.0, C_6H_6O), 84 (87.3, C_4H_6NO), 67 (40.9, C_5H_7), 41 (52.0); **4**: m.p. 110°C; MS (180°C): m/z 240 (M^+, 0.4%), 222 (2.1, $C_{11}H_{14}N_2O_3$), 207 (3.9), 196 (23.3, $C_{10}H_{14}NO_3$), 95 (100%), 84 (25.4), 67 (19.4), 41 (13.4).

11. **1**, MS (210°C): m/z 417.1707 (M^+-2 H_2O, 13.1%, calc. for $C_{24}H_{23}N_3O_4$ 417.1689), 289 (13.5, $C_{19}H_{15}NO_2$), 263 (13.3, $C_{18}H_{17}NO$), 262 (52.5, $C_{18}H_{16}NO$), 261 (17.8, $C_{18}H_{15}NO$), 260 (28.2, $C_{18}H_4NO$), 248 (19.2, $C_{17}H_{14}NO$), 234 (19.8, $C_{16}H_{12}NO$), 221 (18.8, $C_{15}H_{11}NO$), 220 (9.5, $C_{15}H_{10}NO$), 184 (42.1, $C_{12}H_{10}NO$), 172 (13.9, $C_{11}H_{10}NO$), 147 (12.0, C_9H_9NO), 146 (51.2, C_9H_8NO), 133 (26.7, C_8H_7NO), 128 (10.4, $C_5H_8N_2O_2$), 115 (19.9, C_9H_7), 91 (13.7, C_7H_7), 84 (100, C_4H_6NO), 78 (45.5, C_6H_6), 77 (14.6, C_7H_7), 56 (13.9, C_4H_6N).

12. H. Frank, G.J. Nicholson and E. Bayer, J. Chrom. Science **15**, 174 (1977).

13. I. Casser, B. Steffan and W. Steglich, Angew. Chem., in press.

(Received in Germany 10 June 1987)