Oxidation of 3-Hydroxykynurenine. A Reexamination

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Received July 28, 1987

The oxidation mixture of 3-hydroxykynurenine (**1**), treated with aqueous acetic anhydride and, subsequently, with acidic methanol, yields the 1-hydroxy-3-carbomethoxy-5-methoxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)pyrido[3,2-*a*]phenoxazine (**5**), the 1-hydroxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)-5*H*-pyrido[3,2-*a*]-phenoxazin-5-one (**6**), the 1-methoxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)-5*H*-pyrido[3,2-*a*]phenoxazin-5-one (**6a**), the 1,5-dimethoxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)pyrido[3,2-*a*]phenoxazine (**7**) and the 1-methyl-1-{1'-[11-(β-aspartoyl-methyl esterimino)]ethenyl}ketal-1*H*,5*H*-pyrido[3,2-*a*]phenoxazin-5-one (**8**). A probable scheme, for the compound formation, is reported.

J. Heterocyclic Chem., **25**, 1247 (1988).

Introduction, Results and Discussion.

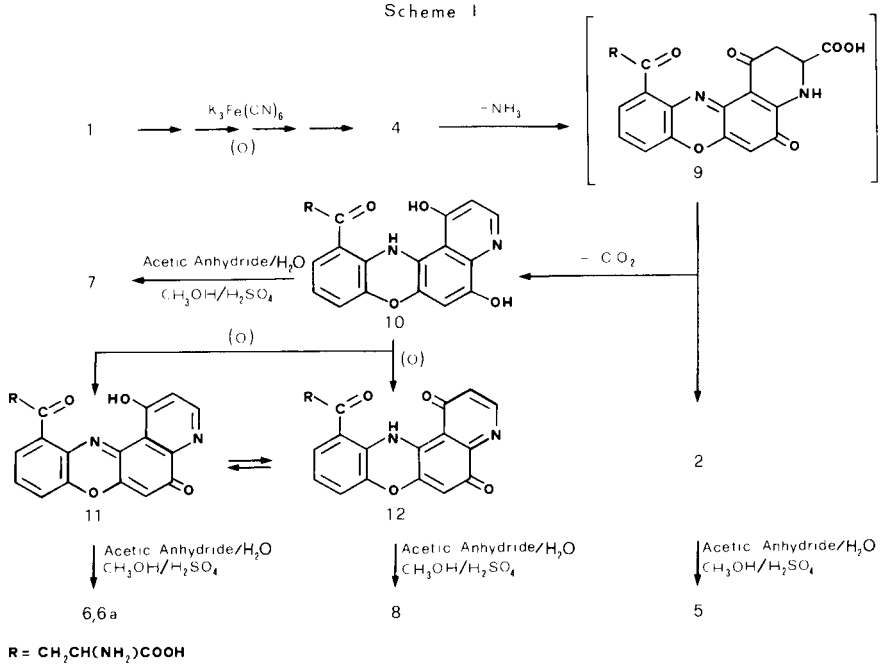
Butenandt and his co-workers [1] identified, from natural sources, four ommatines, arising from oxidation of 3-hydroxykynurenine (**1**), dihydroxanthommatin (**2**) and its derivatives **2a**, **2b**, and xanthommatin (**3**), and isolated **2** by precipitation with sulphurous anhydride from the reaction mixture of **1** with potassium ferricyanide.

From the cephalopod skin, we recently isolated the 1,9-di-(β-aspartoyl-*N*-acetyl-methyl ester)-2-amino-3*H*-phenoxazin-3-one (**4**) [2,3].

The oxidation of **1**, in phosphate buffer at *p*H 6.8 with potassium ferricyanide, was reexamined [4]. Since the obtained pigments were insoluble in organic solvents and non-volatile, *i.e.* non-analyzable with usual spectroscopic (ir, nmr, mass) and chromatographic techniques, they were treated with aqueous acetic anhydride and, subsequently, with methanol-sulphuric acid. In this way some yellow and red pigments, **5**, **6**, **6a**, **7** and **8**, purified on silica gel layers, were identified, on the basis of their spectral (ir, uv, mass, nmr) and chromatographic properties.

The structure of **5** was identified as the 1-hydroxy-3-carbomethoxy-5-methoxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)pyrido[3,2-*a*]phenoxazine. The mass spectrum showed the molecular ion, EI at m/e 509 and, FAB at m/e 510 that must be the result of M + H$^+$. The uv spectrum showed a large maximum, in methanol, at 465 nm characteristic of a phenoxazine system [5]. The ^{1}H nmr showed the characteristic signals attributed to a β-aspartoyl-*N*-acetyl-methyl ester chain. The *N*-proton of the aminoacetyl group appeared as a doublet at δ 6.7 coupled with the multiplet of

Scheme I



the methinic proton at δ 4.96 that was also coupled with the double doublets of the two methylenic protons between δ 3.3-3.7. The *N*-acetyl proton signal appeared as a singlet at δ 2.1. The aromatic protons at C-6, C-8, C-9, C-10 respectively appeared as a singlet at δ 6.5 and as coupled doublet-triplet-doublet between δ 6.4-7.0 according to the proton signals for a phenoxazine structure [5]. The signal at δ 6.3 for the C-2 proton is typical of a pyrido[3,2-*a*]phenoxazine structure. Furthermore, the methoxy and the two carbomethoxy group signals appeared as three singlets between δ 3.7-3.9.

The structure of **6** and **6a** were respectively identified as the 1-hydroxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)-5*H*-pyrido[3,2-*a*]phenoxazin-5-one and the 1-methoxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)-5*H*-pyrido-[3,2-*a*]phenoxazin-5-one. The uv absorption maximum at 430 nm, in methanol, of **6** and **6a** is characteristic of a phenoxazinone system [5]. The mass spectra of the phenoxazinone structures showed not easily explainable peaks. The ^{1}H nmr showed the same typical signal of the β-aspartoyl-*N*-acetyl-methyl ester chain as the compound **5**; only the signal of the two methylene protons appeared as a multiplet at δ 3.7 in the phenoxazinone structures, and as double doublets between δ 3.3-3.7 in the phenoxazine structures. The C-6 proton appeared as a singlet at δ 6.6, characteristic of a quinone structure [6]. The aromatic proton signals at C-8, C-9, C-10 appeared as coupled doublet-triplet-doublet between δ 7.3-7.8, according to the other reported spectral data for phenoxazinone structure. The two coupled doublets at δ 7.0 and δ 8.5 are attributed to the C-2 and C-3 protons, the doublet at lower field was attributed to the C-3 proton analogously to the reported data for the pyridinic structure. Furthermore, the compound **6a** showed a singlet at δ 3.7 of the methoxy group at C-1.

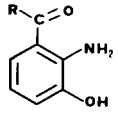
The structure of **7** was identified as the 1,5-dimethoxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)pyrido[3,2-*a*]phenoxazine. The uv absorption maximum at 460 nm and the other spectral data are characteristic for a phenoxazinic structure [5]. The C-2 and C-3 proton signals appeared as two coupled doublets at δ 6.04 and 7.3. The C-2 proton of the compound **5** appeared at a lower field than the compound **7** because of the marked electron-withdrawing effect of the carbomethoxy group at C-3. The C-2 and C-3 protons of the pyrido[3,2-*a*]phenoxazinone structures appeared at a lower field than the analogous protons of the pyrido[3,2-*a*]phenoxazine structures.

The structure of **8** was identified as the 1-methyl-1-{1′-[11-(β-aspartoyl-methyl esterimino)]ethenyl}ketal-1*H*,5*H*-pyrido[3,2-*a*]phenoxazin-5-one. The uv absorption maxium at 436 nm and the ^{1}H nmr spectrum are characteristic of a phenoxazinonec structure [6]. No *N*-proton signal of the β-aspartoyl-*N*-acetyl-methyl ester chain appeared because of the internal ketal formation on C-1 in fact a signal appeared at δ 3.2 typical of methyl protons bonded to an imino-carbon. Furthermore, the methinic proton appeared as a triplet at δ 4.9 only coulped with the multiplet at δ 3.0 of the methylenic group, according to the proposed structure.

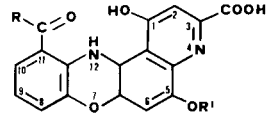
The oxidation reaction of **1**, by ferricyanide, was reported as the nucleophilic attack of **1** on the iminoquinone generated *in situ* [7,8].

Corbett [9], studing the oxidation reaction by ferricyanide between *p*-aminophenol and *m*-aminophenol, established that the reaction is an electrophilic substitution of a *p*-benzosemiquinone monoimine radical cation on *m*-

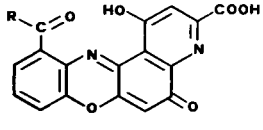
Chart I



1



R^1: 2=H 2a=SO_3H 2b=$C_6H_{10}O_5$



3

R = $CH_2CH(NH_2)COOH$

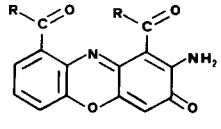


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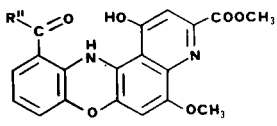
Chart II



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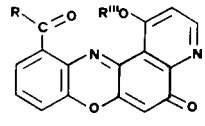
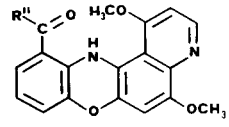


R^{III}: 6 = H 6a = CH_3



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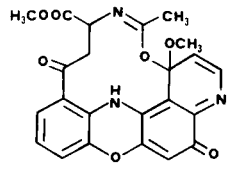

8

R^{II} = $CH_2CH(NHCOCH_3)COOCH_3$

aminophenol aromatic ring.

Furthermore, our study on the oxidation of 4-methyl-2-aminophenol, in buffer by ferricyanide, displays a radical formation [9], according to Corbett.

The reported evidences can support an electrophilic radical mechanism affording the phenoxazinonic ring. Subsequently, a nucleophilic substitution with ammonia elimination could yield the dihydropyridinic ring **9** (Scheme I). After this closure, the compounds **10** and **2** are obtained by an internal proton transfer. The formation of decarboxylated products is also reported during melanin and pheomelanin formation [10,11,12]. Therefore, it seems that the decarboxylation reaction plays an important rôle in the natural pigment formation.

EXPERIMENTAL

The uv spectra were recorded with a Perkin-Elmer 550-S spectrophotometer. The ir spectra were detected in chloroform with a Perkin-Elmer 399 spectrophotometer. The ^1H nmr spectra were recorded with a Varian 200 spectrometer in deuteriochloroform using tetramethylsilane as an internal reference, chemical shifts are given in δ (ppm), s = singlet, d = doublet, t = triplet, m = multiplet; signal attributions were confirmed with the homo-decoupling technique. Mass spectra were determined with a MS 30-AEI spectrometer in EI and with a MS 50 spectrometer in FAB. Melting points were determined with a Kofler apparatus and are uncorrected.

The products were purified on 0.5 mm Whatman PK6F silica gel layers eluted with a benzene-methylene chloride-methanol 50:45:5 v/v mixture (mixture A). The chromatographic purity and Rf were checked on 0.25 mm Whatman PK6F silica gel analytic layers eluted with mix A.

Oxidation of 3-Hydroxykynurenine (**1**).

Three hundred mg of potassium ferricyanide, dissolved in 5 ml of water, were swift added to 100 mg of **1** dissolved in 10 ml of 0.1 *M* phosphate buffer at *p*H 6.8. After the addition of potassium ferricyanide, the solution of **1** was preserved at a temperature of 40° for 15 minutes. After cooling at 5°, acetic acid was added until a brown precipitate was quantitatively obtained and filtrated *in vacuo*. The mother liquor was extracted three times with 20 ml of butanol. The butanol solution, evaporated *in vacuo*, afforded a brown residue that was added to the precipitate. This precipitate was treated with 30 ml of an acetic anhydride-water 50:50 v/v mixture for 18 hours.

The mixture, evaporated *in vacuo* and treated three times with 50 ml of methanol, was treated with 100 ml of a methanol-sulfuric acid 99:1 v/v mixture and heated to reflux for 2 hours.

The solution, cooled to room temperature and neutralized with sodium acetate, was extracted three times with 50 ml of chloroform. The chloroform solution, concentrated *in vacuo* and analyzed on silica gel layers eluted with mixture A, afforded five coloured products: **5**, **6**, **6a**, **7** and **8**.

1-Hydroxy-3-carbomethoxy-5-methoxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)pyrido[3,2-*a*]phenoxazine (**5**).

Compound **5** was obtained as red crystals of mp 210-215° and Rf 0.38 in mixture A; ir (chloroform): 3420-3200 (NH, OH), 1740 ($COOCH_3$), 1660 (C=O) cm^{-1}; uv (methanol): λ max (log ε) 465 nm (3.7), 380 nm (3.5); ^1H nmr (deuteriochloroform): δ 2.1 (s, 3H, CH_3-CO-), 3.4-3.6 (dd, 2H, $-CH_2$-CO-), 3.7 (s, 3H, CH_3O-), 3.8 (s, 3H, CH_3O-), 3.9 (s, 3H, CH_3O-), 4.9 (m, 1H, -C*H*-NH-), 6.3 and 6.5 (1H, 1H, two s attributed to protons 2 and 6), 6.4 (d + t, 2H, aromatic), 6.9 (d, 1H, -N*H*-CH-), 7.0 (d, 1H, aromatic), ms: (EI) m/e 509 (M^+), 464, 450 (100%), 375; (FAB) 510 ($M+H^+$).

Anal. Calcd. for $C_{25}H_{23}N_3O_9$: C, 59.40; H, 4.86; N, 8.80. Found: C, 59.25; H, 4.26; N, 9.35.

1-Hydroxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)-5*H*-pyrido[3,2-*a*]phenoxazin-5-one (**6**).

From the reaction mixture yellow crystals of mp 258-261° and Rf 0.26 (mixture A) were isolated; ir (chloroform): 3420-3200 (NH, OH), 1740 ($COOCH_3$), 1660 (C=O) cm^{-1}; uv (methanol): λ max (log ε) 430 nm (3.95), 350 nm (shoulder); ^1H nmr (deuteriochloroform): δ 2.1 (s, 3H, CH_3-CO-), 3.7 (m, 2H, $-CH_2$-CO-), 3.8 (s, 3H, CH_3O-), 4.96 (m, 1H, -C*H*-NH-), 6.6 (s, 1H, quinonic), 6.7 (d, 1H, -N*H*-CH-), 7.2 (d, 1H, -C=CH-), 7.56 (d, 1H, aromatic), 7.64 (t, 1H, aromatic), 7.78 (d, 1H, aromatic), 8.76 (d, 1H, -N=CH-).

Anal. Calcd. for $C_{22}H_{17}N_3O_7$: C, 60.69; H, 3.94; N, 9.65. Found: C, 60.52; H, 3.98; N, 9.71.

1-Methoxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)-5*H*-pyrido[3,2-*a*]phenoxazin-5-one (**6a**).

From the reaction mixture **6a** was obtained as yellow crystals of mp 258-261° and Rf 0.27 (mixture A); ir (chloroform): 3420-3200 (NH), 1740 ($COOCH_3$), 1670-1650 (C=O) cm^{-1}; uv (methanol): λ max (log ε) 430 nm (3.95), 350 nm (shoulder); ^1H nmr (deuteriochloroform): δ 2.1 (s, 3H, CH_3-CO-), 3.7 (s, 3H, CH_3O-), 3.7 (m, 2H, $-CH_2$-CO-), 3.8 (s, 3H, CH_3O-), 4.96 (m, 1H, -C*H*-NH-), 6.0 (s, 1H, quinonic), 6.7 (d, 1H, -N*H*-CH-), 7.1 (d, 1H, -C=CH-), 7.6 (d, 1H, aromatic), 7.68 (t, 1H, aromatic), 7.8 (d, 1H, aromatic), 8.8 (d, 1H, -N=CH-).

Anal. Calcd. for $C_{23}H_{19}N_3O_7$: C, 61.47; H, 4.26; N, 9.35. Found: C, 61.13; H, 4.23; N, 9.60.

1,5-Dimethoxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)pyrido[3,2-*a*]phenoxazine (**7**).

From the compound mixture **7** was isolated as orange crystals of mp 215-221° and Rf 0.23 (mixture A); ir (chloroform): 3420-3200 (NH), 1740 ($COOCH_3$), 1660 (C=O) cm^{-1}; uv (methanol): λ max (log ε) 460 nm (3.7), 375 nm (3.3); ^1H nmr (deuteriochloroform): δ 2 (s, 3H, CH_3O-), 3.4-3.6 (dd, 2H, $-CH_2$-CO-), 3.75 (s, 3H, CH_3O-), 3.85 (s, 3H, CH_3O-), 3.92 (s, 3H, CH_3O-), 4.96 (m, 1H, -C*H*-NH-), 6.04 (d, 1H, -C=CH-), 6.41 (s, 1H, aromatic), 6.51 (d + t, 2H, aromatic), 7.0 (d, 1H, -N*H*-CH-), 7.15 (d, 1H, aromatic), 7.3 (d, 1H, -N=CH-); ms: m/e 465 (M^+), 390, 346, 331 (100%).

Anal. Calcd. for $C_{24}H_{23}N_3O_7$: C, 61.93; H, 4.98; N, 9.03. Found: C, 61.73; H, 5.02; N, 9.05.

1-Methyl-1-{1'-[11-(β-aspartoyl-methyl esterimino)]ethenyl}ketal-1*H*,5*H*-pyrido[3,2-*a*]phenoxazin-5-one (**8**).

Compound **8** was obtained as yellow crystals of mp 247-249° and Rf

0.29 (mixture A); ir (chloroform): 3420-3300 (NH), 1740 ($COOCH_3$), 1670-1650 (C=O) cm^{-1}; uv (methanol): λ max (log ε) 436 nm (3.9), 390 nm (shoulder); ^1H nmr (deuteriochloroform): δ 3.0 (m, 2H, $-CH_2-CO-$), 3.2 (s, 3H, $-N=C-CH_3$), 3.5 (s, 3H, CH_3O-), 3.7 (s, 3H, CH_3O-), 4.9 (t, 1H, $-CH_2-CH-$), 6.6 (s, 1H, quinonic), 7.0 (d, 1H, $-C-CH=$), 7.4 (d, 1H, aromatic), 7.63 (t, 1H, aromatic), 7.82 (d, 1H, aromatic), 8.5 (d, 1H, $=CH-N=$).

Anal. Calcd. for $C_{23}H_{19}N_3O_7$: C, 61.47; H, 4.26; N, 9.35. Found: C, 61.32; H, 4.19; N, 9.51.

Acknowledgement.

This work was supported by a grant from Ministero della Pubblica Istruzione. Mass spectral data were provided by "Servizio di Spettroscopia di Massa del CNR dell'Università di Napoli".

REFERENCES AND NOTES

[1] A. Butenandt and W. Schäfer, "Recent Progress in the Chemistry of Natural and Synthetic Colouring Matters and Related Fields", Academic Press, New York, 1962, p 13.

[2] A. Bolognese, C. Piscitelli and G. Scherillo, *Rc. Accad. Sci. Mat. Fis.*, Napoli, **52/I**, 41 (1985).

[3] A. Bolognese, C. Piscitelli and G. Scherillo, *Rc. Accad. Sci. Mat. Fis.*, Napoli, **52/I**, 53 (1985).

[4] A. Butenandt, U. Schiedt, E. Biekert and R. I. T. Cromartie, *Ann. Chem.*, **590**, 75 (1955).

[5] W. Schäfer, I. Geyer and H. Schlude, *Tetrahedron*, **28**, 3811 (1972).

[6] A. Bolognese, C. Piscitelli and G. Scherillo, *J. Org. Chem.*, **48**, 3649 (1983).

[7] A. Bolognese, C. Piscitelli and G. Scherillo, *Rc. Accad. Sci. Mat. Fis.*, Napoli, **51**, 415 (1983).

[8] "The Chemistry of the Quinonoid Compounds", S. Patai, ed, John Wiley and Sons Ltd., 1974, p 877.

[9] K. C. Brown, J. F. Corbett and R. Labinson, *J. Chem. Soc., Perkin Trans. II*, 1292 (1978).

[10] R. A. Nicolaus, "Melanins", E. Lederer, ed, Hermann, Paris, 1968.

[11] A. Napolitano, F. Chioccara and G. Prota, *Gazz. Chim. Ital.*, **115**, 357 (1985).

[12] G. Prota and R. A. Nicolaus, *Gazz. Chim. Ital.*, **97**, 665 (1967).