Orig Life Evol Biosph (2011) 41:317–330
DOI 10.1007/s11084-011-9236-3

PREBIOTIC CHEMISTRY

The Effects of Borate Minerals on the Synthesis of Nucleic Acid Bases, Amino Acids and Biogenic Carboxylic Acids from Formamide

Raffaele Saladino · Maurizio Barontini · Cristina Cossetti · Ernesto Di Mauro · Claudia Crestini

Received: 20 December 2010 / Accepted: 16 February 2011 /
Published online: 19 March 2011

Abstract The thermal condensation of formamide in the presence of mineral borates is reported. The products afforded are precursors of nucleic acids, amino acids derivatives and carboxylic acids. The efficiency and the selectivity of the reaction was studied in relation to the elemental composition of the 18 minerals analyzed. The possibility of synthesizing at the same time building blocks of both genetic and metabolic apparatuses, along with the production of amino acids, highlights the interest of the formamide/borate system in prebiotic chemistry.

Keywords Prebiotic chemistry · Borates · Formamide · Nucleic acid bases · Biogenic carboxylic acids

Introduction

The role of borate minerals in the synthesis of sugar from formaldehyde and glycolaldehyde is receiving increasing attention. Pivotal studies showed that boric acid (H_3BO_3) and mineral borates ulexite [$NaCaB_5O_6(OH)_6 \cdot 5H_2O$], kernite [$Na_2B_4O_6(OH)_2 \cdot 3H_2O$] and colemanite ($CaB_3O_4(OH)_3 \cdot H_2O$) catalyze the formation and stabilization of pentoses in the cyclic furanose form (Prieur 2001; Ricardo et al. 2004; Scorei and Cimpoiaşu 2006). In particular, the stabilization of ribose, one of the few sugars supporting the Watson-Crick molecular recognition processes essential for Darwinian evolution, was observed. Due to

R. Saladino (✉) · M. Barontini
Dipartimento di Agrobiologia ed Agrochimica, Università della Tuscia, Via S. Camillo de Lellis s.n.c., 01100 Viterbo, Italy
e-mail: saladino@unitus.it

C. Cossetti · E. Di Mauro
Dipartimento di Biologia e Biotecnologie Charles Darwin, Università "Sapienza", Rome, Italy

C. Crestini
Scienze e Tecnologie Chimiche, Università Tor Vergata, Rome, Italy

the high electronic deficiency of boron, borate forms complexes with the vicinal 1,2-diol moiety in key intermediates of the formose condensation sequence leading to sugars, such as glyceraldehyde. These complexes provide selectivity to the reaction, preventing the nucleophilic character of glyceraldehyde, while retaining its electrophilicity towards enediolate glycolaldehyde condensing units. Moreover, at the end of the reaction, borate yields a complex with ribose which is more stable than ribose itself. Among the chemical precursors studied in prebiotic chemistry, formamide (NH_2CHO) provides a unitary framework for the synthesis of nucleic acids precursors, amino acids, and amino-sugar derivatives under simple experimental conditions compatible with the primitive Earth (Saladino et al. 2004; Saladino et al. 2006; Saladino et al. 2009).

A possible relationship between formamide and borate minerals has been discussed by Benner et al. (2006) in the context of the "water paradox" stating that polymerization of biomolecules requiring release of water are, in water, thermodynamically disfavoured. During dry-wet cycles presumably fostering the formation of oligonucleotides, two processes can occur: on one hand the formation of borate evaporites, mineral deposits that emerge from evaporating water, are favoured by the presumptive occurrence of evaporitic basins, whether continental or marine on a prebiotic Earth (as discussed in Anovitz and Grew 2002, and in Grew et al. 2011). On the other the concentration of formamide, which is characterized by a boiling point of 210°C in the absence of eutectic with water. In this scenario, both the formation of ribose catalyzed by borates and polymerization processes of nucleotides in formamide as thermodynamically favourable environment, are hypothesized (Benner et al. 2006). Based on our previous results on formamide chemistry, recently reviewed (Saladino et al. 2007), we reasoned that formamide could also oligomerize in the presence of mineral borates. No previous studies are available on the role of borates in the prebiotic synthesis of biomolecules other than sugars. We report that borates, whose structures encompass a large variety of elemental compositions and chemical properties, catalyze the synthesis of nucleic acid bases, amino acids and biogenic carboxylic acids from formamide. The yield and selectivity of these reactions was found to depend on the mineral used in the condensation. These data, in connection with the recent use of H_3BO_3 as a catalyst for the polymerization of amino acids (Kolitz et al. 2009), further support a major role of borate minerals in prebiotic processes.

Material and Methods

Formamide (Fluka, >99%) was used without further purification. Gas-chromatography mass-spectrometry (GC-MS) analyses were performed with a HP5890II gas chromatograph and a Shimadzu GC-MS QP5050A with a Variant CP8944 column (WCOT fused silica, film thickness 0.25 μm, stationary phase VF-5 ms, $Ø_i$ 0.25 mm, lenght 30 m). Samples were analyzed after treatment with *N*,*N*-bis-trimethylsilylacetamide in pyridine using standard procedures and betulin as internal standard for quantitative analyses. When necessary, purifications were performed on chromatography columns packed with Merck silica gel, 230–400 mesh for flash technique. Nuclear Magnetic Resonance spectra (^{1}H-NMR and ^{13}C-NMR) were recorded on a Bruker (200 MHz) spectrometer and are reported in δ (ppm) value. The borate minerals were obtained from Ezio Curti (ezio.curti@gmail.it), former provider and consultant of the collection of the minerals of the Department of Mineralogy (University of Rome "Sapienza", Italy).

The provenance of the minerals is: axinite-(Mn), Harz Mountain, Germany; borax, Sigma-Aldrich; canavesite, Brosso Mine, Canavese, Piedmont, Italy; chambersite,

Venice Salt Dome, Plaque Mine Paris, Louisiana USA; colemanite, Furnace Creek, Death Valley, California USA; dravite, Drava River, Austria; dumortierite, Soavina Mine, Madagascar; elbaite, Jonas, Minas Gerais, Brazil; hydroboracite, Boron, Kern County, California USA; kernite, Boron, Kern County, California USA; kornerupine, Itrongay, Madagascar; kurnakovite, Boron, Kern County, California USA; ludwigite, Corcolle querry, Rome, Latium, Italy; painite, Ohngaing, Myanmar; rhodizite, Manandona Valley, Madagascar; schorl, Galileia, Minas Gerais, Brazil; ulexite, Boron, Kramer District, Kern County, California USA; vonsenite, Vetralla, Latium Viterbo, Italy.

Reactions were performed with homogenous material isolated under the microscope, washed twice (with ethanol and analytic grade distilled water), air dried, then manually ground in a ceramic mortar. Isolation of homogeneous material under the microscope was performed mechanically, selecting the fragments of the wanted mineral resulting from a previously performed crushing procedure, discarding the spurious components. Samples of crushed materials are available upon request, except for canavesite, chambersite and kornerupine, which may be purchased at Dakota Matrix Minerals (www.dakotamatrix.com) and John Betts Fine Minerals (www.johnbetts-fineminerals.com).

Formamide Condensation. General Procedure

Formamide **1** (5.7 g, 5 mL, 0.12 mmol) was heated at 160°C for 48 h in the presence of the appropriate borate mineral (2.0% in weight). At the end of the reaction the mixture was cooled and the residual mineral was recovered by centrifugation with a Heraeus Biofuge 15 apparatus, washed with a little amount of neat formamide (0.5 ml) and separated again by centrifugation. The organic phase was evaporated under high vacuum to yield a dark crude that was analyzed by GC-MS analyses. Selected mass spectrometric data of compounds **2**–**17** are reported in Table 1. In the case of kernite and colemanite, which are characterized by a high solubility in formamide, the GC-MS analysis was performed directly on the crude of the reaction.

In the case of axinite-(Mn) the reaction was repeated in higher amount and the crude, after purification (flash-chromatography), was analyzed by NMR techniques.

Selected spectroscopic data:

Uracil (**4**): m.p. >300°C; ^{1}H NMR (DMSO-d_6) δ ppm: 11.0 (3H, s, NH, NH_2), 7.38 (1H, m, CH), 5.50 (1H, m, CH). ^{13}C NMR (DMSO-d_6) δ ppm: 164.10 (C=O), 153.10 (C=O), 142.10 (2xCH).

Isocytosine (**5**): m.p. 275°C; ^{1}H NMR (DMSO-d_6) δ ppm: 7.42 (3H, s, NH, NH_2), 7.20 (1H, d, J=7.43 MHz, CH), 5.62 (1H, d, J=7.43 MHz, CH). ^{13}C NMR (DMSO-d_6) δ ppm: 171.07 (C=O), 150.06 (C), 131.79 (CH), 110.2 (CH).

Cytosine (**6**): m.p. >300°C; ^{1}H NMR (DMSO-d_6) δ ppm: 7.30 (1H, m, CH), 7.10 (3H, s, NH, NH_2), 5.70 (1H, m, CH). ^{13}C NMR (DMSO-d_6) δ ppm: 168.10 (C=O), 158.20 (C), 142.11 (CH), 93.10 (CH).

Purine (**7**): m.p. 214–217°C; ^{1}H NMR (DMSO-d6) δ ppm: 9.12 (1H, s, CH), 8.90 (1H, s, CH), 8.61 (1H, s, CH). ^{13}C NMR (DMSO-d6) δ ppm: 154.66 (C), 152.0 (C), 146.10 (CH), 145.51 (CH), 130.34 (C).

Adenine (**8**): m.p. >360°C; ^{1}H NMR (D_2O+DCl) δ ppm: 8.60 (1H, s, CH), 8.50 (1H, s, CH). ^{13}C NMR (D_2O+DCl) δ ppm: 152.71 (C), 150.96 (C), 147.43 (CH), 145.76 (CH), 121.70 (C).

Table 1 Selected mass Gas-Chromatography Mass-Spectroscopy data (GC-MS) of condensation products **2**–**17**[a]

Products	*m/z* (%)
3-Hydroxy pyridine (**2**)	167 (10) [M], 152 (100) [M-CH_3], 137 (6) [M-$(CH_3)_2$], 122 [M-$(CH_3)_3$]
2(1H)pyrimidinone (**3**)[b]	168 (25) [M], 153 (100) [M-CH_3], 123 (3) [M-$(CH_3)_3$]
Uracil (**4**)[c]	256 (35) [M], 241 (100) [M-CH_3], 225 (15) [M-$(CH_3)_2$], 182 (7)) [M-$Si(CH_3)_3$, 142 (70), 113 (55)
Isocytosine (**5**)[d]	327 (18) [M], 312 (100) [M-CH_3], 282 (9) [M-$(CH_3)_3$], 255 (6) [M-H-$Si(CH_3)_3$], 240 (7) [M-H-$Si(CH_3)_3$-CH_3], 182 (2) [M-2$Si(CH_3)_3$]
Cytosine (**6**)[c]	255 (49) [M], 254 (100) [M-H], 240 (72) [M-CH_3], 182 (5) [M-H-$Si(CH_3)_3$]
Purine (**7**)[b]	192 (79) [M], 177 (100) [M-CH_3], 120 (10) [M-$Si(CH_3)_3$]
Adenine (**8**)[c]	279 (27) [M], 264 (100) [M-CH_3], 249 (1) [M-$(CH_3)_2$], 192 (17) [M-$Si(CH_3)_3$]
Carbodiimide (**9**)[c]	186 (14) [M], 171 (100) [M-CH_3], 141 (4) [M-$(CH_3)_3$], 113 (2) [M-$Si(CH_3)_3$], 98 (11) [M-$Si(CH_3)_3$-CH_3], 83 (2) [M-$Si(CH_3)_3$-$(CH_3)_2$]
Lactic acid (**10**)[c]	219 (6) [M-CH_3], 190 (14) [M-CO_2], 147 (71) [M-$Si(CH_3)_3$-CH_3], 133 (7), 117 (76) [M-$Si(CH_3)_3$-$(CH_3)_3$].
Lactic acid dimer (**11**)[c]	289 (7) [M-CH_3], 274 (5) M-2xCH_3], 186 (12) [M-CO-$OSi(CH_3)_3$], 158 (70)
Oxalic acid (**12**)[c]	219 (3) [M-CH_3], 189 (5) [M-$(CH_3)_3$], 147 (78) [M-$Si(CH_3)_3$-CH_3], 117 (1) [M-$Si(CH_3)_3$-3CH_3], 73 (100) [M-2$Si(CH_3)_3$-O]
Glycolic acid **(13)**[c]	205 (8) [M-CH_3], 190 (1) [M-$(CH_3)_2$], 148 (10) [M-$Si(CH_3)_3$], 147 (74) [M-H-$Si(CH_3)_3$], 133 (9) [M-CH_3-$Si(CH_3)_3$], 117 (4) [M-H-$(CH_3)_2$-$Si(CH_3)_3$], 103 (5) [M-$(CH_3)_3$-$Si(CH_3)_3$]
Pyruvic acid (**14**)	144 (10) [M-CH_3], 116 [M- CH_3-CO], 87 (56) [M- $Si(CH_3)_3$]
Glyoxylic acid (**15**)[c]	221(15) [M-CH_3], 191 (81) [M-$(CH_3)_3$], 149 (5) [M-$Si(CH_3)_3$-CH_3], 147 (57) [M-$Si(CH_3)_3$-OH], 74 (9) [M-2$Si(CH_3)_3$-H_2O], 73 (100)
N-formylglycine (**16**)[c]	246 (5) [M-H], 231 (7) [M-CH_4], 174 (12) [M-H-$Si(CH_3)_3$], 129 (11) [M-H-$Si(CH_3)_3$-$(CH_3)_3$], 101 (11) [M-H-2x$Si(CH_3)_3$], 73 (100) [M-H-2x$Si(CH_3)_3$-CO]
N-formylalanine (**17**)	188 (6) [M], 173 (12)) [M-(CH_3)], 159 (20) [M-HCO], 100 (35)[M-O $Si(CH_3)_3$]

[a] Mass spectroscopy was performed by using a GC-MS QP5050A. Samples were analyzed after treatment with *N,N*-bis-trimethylsilyltrifluoroacetamide and pyridine. The abundance of peak is reported in parenthesis. [b] Product analyzed as the monosilyl derivative; [c] Product analyzed as the bis-silyl derivative; [d] Product analyzed as the tris-silyl derivative. [e] Analytical tools in experimentals

Lactic acid (**10**):): m.p. 52–45°C; ^{1}H NMR ($CDCl_3$) δ ppm: 4.31 (1H, m, CH), 1.38 (3H, m, CH_3) . ^{13}C NMR ($CDCl_3$) δ ppm: 178.10 (C=O), 62.12 (CH), 68.49 (CH), 20.0 (CH_3).

Lactic acid dimer (**11**): oil; ^{1}H NMR ($CDCl_3$) δ ppm: 5.11 (1H, m, CH), 4.49 (1H, m, CH), 1.58 (3H, m, CH_3), 1.40 (3H, m, CH_3) . ^{13}C NMR ($CDCl_3$) δ ppm: 175.20 (C=O), 171.91 (C=O), 72.43 (CH), 68.49 (CH), 20.13 (CH_3), 18.40 (CH_3).

Oxalic acid (**12**): m.p. 189.5°C; ^{13}C NMR (D_2O) δ ppm: 161.80 (C=O).

Pyruvic acid (**14**): oil; ^{1}H NMR ($CDCl_3$) δ ppm: 8.70 (1H, br. s, OH), 2.51 (3H, s, CH_3) . ^{13}C NMR ($CDCl_3$) δ ppm: 194.10 (C=O), 161.80 (C=O), 26.13 (CH_3).

Glyoxylic acid (**15**): m.p. 49–52°C; ^{1}H NMR ($CDCl_3$) δ ppm: 9.66 (1H, br. s, OH), 9.42 (1H, s, CH) . ^{13}C NMR ($CDCl_3$) δ ppm: 192.57 (HCO), 165.55 (COOH).

N-formylglycine (**16**): m.p. 149–151°C; ^{1}H NMR (DMSO-d_6) δ ppm: 8.22 (1H, br. s, OH), 8.06 (1H, s, C*H*O), 3.86-3.78 (2H, m, CH_2). ^{13}C NMR (DMSO-d_6) δppm: 174.62 (C), 164.33 (C), 41.53 (CH_2).

Results and Discussion

Heat-driven formamide condensation experiments were performed in the presence of a large panel of borate minerals whose elemental composition is reported in Table 2. These minerals are arranged in different groups on the basis of the Dana's classification rules depending on the type of boron-oxygen radical (e.g., the degree of association of elementary structural units such as B-triangles and B-tetrahedra) and on their chemical and physical properties (Gaines et al. 1997). The following selected Dana's groups were: a) Hydrates of borates containing hydroxyl or halogen moieties (group A), borax (sodium tetraborate), colemanite, hydroboracite, kernite, kurnakovite and ulexite; b) Anhydrous

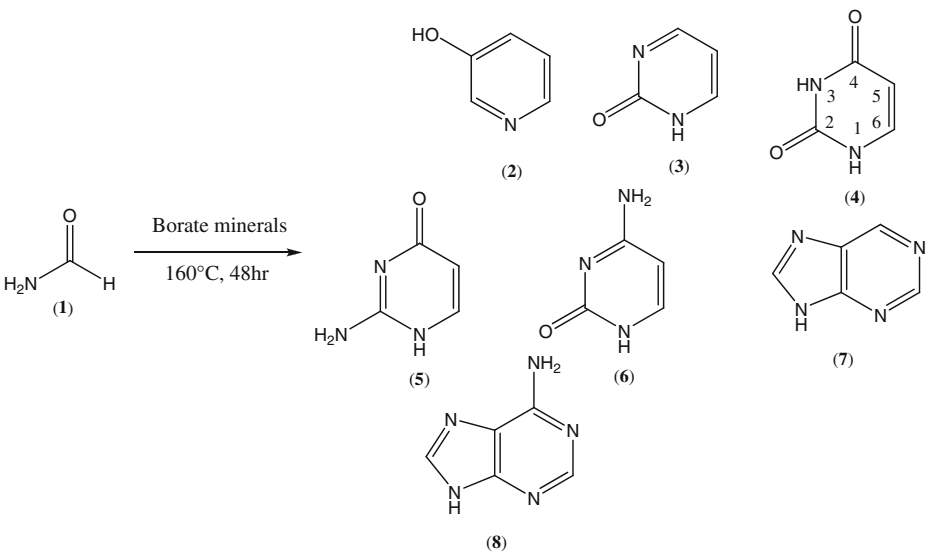
Table 2 Borate minerals used in formamide prebiotic chemistry

Mineral	Elemental composition	Dana's Classification Group
Borax	$Na_2B_4O_7 \cdot 10\ (H_2O)$	A[a]
Colemanite	$CaB_3O_4(OH)_3 \cdot H_2O$	A
Hydroboracite	$CaMgB_6O_8(OH)_6 \cdot 3\ (H_2O)$	A
Kernite	$Na_2B_4O_6(OH)_2 \cdot 3\ (H_2O)$	A
Kurnakovite	$MgB_3O_3(OH)_5 \cdot 5\ (H_2O)$	A
Ulexite	$NaCaB_5O_6(OH)_6 \cdot 5\ (H_2O)$	A
Chambersite	$Mn_3B_7O_{13}Cl$	B[b]
Hambergite	$Be_2(BO_3)(OH)$	B
Ludwigite	$Mg_2Fe^{3+}BO_5$	B
Rhodizite	$(K,Cs)Al_4Be_4(B,Be)_{12}O_{28}$	B
Vonsenite	$Fe_2{}^{2+}Fe^{3+}BO_5$	B
Dravite	$NaMg_3Al_6(BO_3)_3Si_6O_{18}(OH)_4$	C[c]
Dumortierite	$Al_{6.9}(BO_3)(SiO_4)_3O_{2.5}(OH)_{0.5}$	C
Elbaite	$NaLi_{2.5}Al_{6.5}C_3Si_6O_{18}(OH)_4$	C
Kornerupine	$(Mg,Fe^{+2})_4(Al,Fe^{3+})_6(SiO_4,BO_4)_5(O,OH)_2$	C
axinite-(Mn)	$Ca_2Mn^{2+}Al_2(BO_3)Si_4O_{12}(OH)$	C
Schorl	$NaFe^{+2}Al_6\ (BO_3)_3Si_6O_{18}(OH)_4$	C
Canavesite	$Mg_2(CO_3)(HBO_3) \cdot 5\ (H_2O)$	D[d]
Painite	$CaZrB(Al_9O_{18})$	E[e]
Boric anhydride	B_2O_3	none
Sodium perborate	$NaBO_3 \cdot 4\ (H_2O)$	none

[a] Hydrates of borates containing hydroxyl or halogen moieties. [b] Anhydrous borates. [c] Borosilicates. [d] Borocarbonates. [e] Multiple oxides

borates containing hydroxyl or halogen moieties (group B), chambersite, hambergite, ludwigite, rhodizite and vonsenite; c) Borosilicates (group C), dravite, dumortierite, elbaite, kornerupine, axinite-(Mn) and schorl; d) Borocarbonates (group D) represented by canavesite; e) Multiple oxides (group E) represented by painite. Synthetic boron derivatives boron anhydride and sodium perborate were also used for comparison. Borax, a mineral occurring commonly on extant Earth, abundant in evaporates in desert environments, is particularly relevant in a prebiotic perspective. An alternative system for grouping the boron minerals has also been proposed (Hawthorne et al. 2002), organizing them according to the hierarchical organization based on fundamental building blocks of the structures. We will not go into the differences in these classification systems here. We analyze a large panel of boron minerals and refrain from indicating which (or which sub-class of) boron minerals might have played the most relevant role in prebiotic chemistry. Important discussion on abundance, occurrence, origin and age of these minerals are in Dunn (1995), Ciriotti et al. (2009), Grew and Hazen (2010), Grew et al. (2011).

As a general procedure, the syntheses were performed by heating pure formamide **1** (5 ml; 0.126 mol) at 160°C for 48 h in the presence of catalytic amounts of the appropriate mineral (2% in weight). Given the uncertainty of the stoichiometry of the reaction, the yield was reported as mg of product formed *per* gram of formamide. We focused on the characterization of the more abundant products by gas chromatography/mass spectrometry analysis (GC-MS analysis) by comparison with authentic samples and, in a selected case [axinite-(Mn)], by Nuclear Magnetic Resonance analysis (^{1}H-NMR and ^{13}C-NMR) on purified sample. The condensation of **1** in the absence of borate minerals afforded purine as the only recovered product (c.a. 34.0 mg per gram of formamide). In the presence of borate minerals a large panel of compounds relevant for the origin of the genetic and metabolic apparatuses was obtained including components of nucleic acids and heterocycle derivatives **2**–**8** (Scheme 1, Table 3), carbodiimide **9**, carboxylic acids **10**–**15** and amino acid derivatives **16**–**17** (Scheme 2, Table 4).



Scheme 1 Synthesis of nucleic acid and heterocycle derivatives from formamide and mineral borates

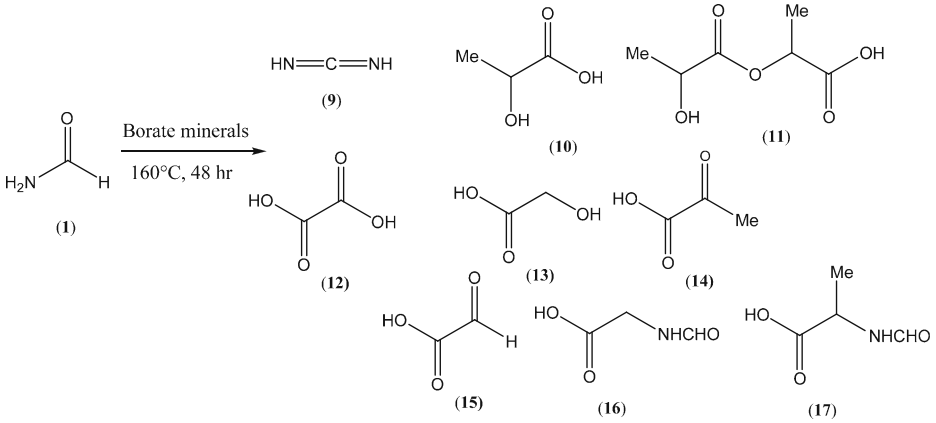
Table 3 Quantitative profile of compounds **2**–**8** produced by the condensation of formamide and borate minerals[a]

Entry	Catalyst	Yield [mg g^{-1}][b]						
		(2)	**(3)**	**(4)**	**(5)**	**(6)**	**(7)**	**(8)**
1	Borax[c]	0.005	0.026	n.d.	0.001	0.002	0.80	n.d.
2	Colemanite[c]	0.004	0.046	0.004	0.007	0.003	1.94	n.d.
3	Hydroboracite[c]	0.002	0.033	n.d.	0.009	n.d.	0.7	n.d.
4	Kernite[c]	<0.001	<0.001	n.d.	<0.01	n.d.	0.013	n.d.
5	Kurnakovite[c]	0.02	0.079	n.d.	0.023	n.d.	0.81	n.d.
6	Ulexite[c]	0.003	0.052	<0.001	0.003	<0.001	1.80	n.d.
7	Chambersite[d]	0.002	0.012	0.005	0.002	n.d.	0.074	0.023
8	Hambergite[d]	0.006	0.180	n.d.	0.028	0.058	0.56	0.110
9	Ludwigite[d]	0.061	n.d.	n.d.	0.003	n.d.	n.d.	0.022
10	Rhodizite[d]	<0.001	0.14	n.d.	0.011	n.d.	0.52	0.120
11	Vonsenite[d]	0.001	0.114	0.007	0.003	0.008	2.50	0.660
12	Dravite[e, l]	0.070	0.990	0.402	0.73	n.d.	19.29	0.016
13	Dumortierite[e]	0.003	0.140	0.002	n.d.	n.d.	0.915	n.d.
14	Elbaite[e]	0.007	0.130	0.046	<0.001	n.d.	0.99	<0.001
15	Kornerupine[e, h]	0.004	0.127	0.003	n.d.	n.d.	0.45	n.d.
16	axinite-(Mn)[e]	0.05	0.20	0.063	0.02	0.05	0.56	0.010
17	Schorl[e]	0.005	0.67	0.012	n.d.	0.03	0.87	n.d.
18	Canavesite[f, i]	<0.001	0.010	n.d.	0.002	n.d.	0.241	n.d.
19	Painite[g, h]	0.006	0.40	0.007	n.d.	n.d.	n.d.	0.026
20	Boric anhydride	0.003	0.112	n.d.	0.008	0.003	1.30	n.d.
21	Na perborate	0.003	0.021	n.d.	0.002	0.049	0.79	n.d.
22	None	n.d.	n.d.	n.d.	n.d.	n.d.	34.0	n.d.

[a] Products were identified by comparison of their retention time and mass-spectra with those of authentic samples. [b] Quantitative evaluations were performed by capillary gas-chromatographic analysis (GC) as described in the experimental section. Because of the uncertainty of the moles of formamide involved in the synthesis of the recovered products, the yields are reported as mg of product formed per gram of formamide. [c] Group A. For Dana's classification see Table 1. [d] Group B. [e] Group C. [f] Group D. [g] Group E. [h] Traces of isocyanate. [i] In this case parabanic acid (0.014 mg/gr of formamide) was also detected. [l] Traces of diaminomaleodinitrile (DAMN).

Synthesis of Nucleic Acid and Heterocycle Derivatives

The synthesis of nucleobases from formamide requires the generation "in situ" of HCN and of others low molecular weight compounds, such as formaldehyde and ammonium formate, that are involved in the condensation process (Saladino et al. 2005b). Two main reaction pathways are suggested for the condensation. Yamada and Okamoto (1972) described the formation of a substituted 5,6-dihydro pyrimidinone derivative as a common intermediate for nucleobases, produced by a sequential condensation of formamide and HCN. In this latter case, the reduction of two exocyclic imino moieties on the pyrimidine ring was a specific redox step to obtain the C-5/C-6 double bond in pyrimidine nucleobases. As an alternative, purines can be synthesized by a multi-steps process involving HCN oligomers, 5-imidazole carbonitrile and 5-imidazole carboxyamide (not shown), that are also key



Scheme 2 Synthesis of biogenic carboxylic acids and amino acid derivatives from formamide and mineral borates

intermediate in the polymerization of HCN (Sanchez et al. 1966; Voet and Schwartz 1983; Schwartz and Bakker 1989; Saladino et al. 2007).

Mineral borates catalyzed the synthesis of both purine and pyrimidine nucleobases. Uracil **4**, cytosine **6** and adenine **8** are DNA and RNA nucleobases, while isocytosine [2-aminopyrimidinin-4(3*H*)-one, iC] **5**, that is not a component of nucleic acids, recognizes **6** through a Watson-Crick hydrogen bonding pattern similar to that of the guanine/cytosine pair (Zhanpeisov and Leszczynski 1999). Compound **5** also recognizes guanine by a reversed Watson-Crick interaction (Gupta et al. 2004). 2(1*H*)-pyrimidinone **3** was previously synthesized from formamide in the presence of iron sulphur and iron-copper sulphur minerals (Saladino et al. 2008), such as pyrite (FeS_2) and pyrrhotite ($Fe_{(1-x)}S$), key reagents in the Wächtershäuser model of the chemoautotrophic origin of the secondary metabolism (Wächtershäuser 1988; Wächtershäuser 1990; Wächtershäuser 1992). Compound **3** is an alternative to natural pyrimidine nucleobases in a pre-RNA world due to reported synthesis of the corresponding pyrimidinone ribonucleoside in a plausible prebiotic scenario (Bean et al. 2007). The 3-hydroxy pyrimidine **2** was not previously detected in formamide-based syntheses, while the 4(3*H*)-pyrimidinone (4-hydroxy pyrimidine in the tautomeric form), a minor component in the Murchison meteorite, was obtained from formamide in the presence of cosmic dust analogues of terrestrial olivines (Saladino et al. 2005a).

Irrespective of the nature of the borate mineral, purine **7** was recovered as the main reaction product. Adenine **8** was synthesized in the presence of anhydrous borates (Table 3, entries 7–11) and of painite (Table 3, entry 19), but not in the presence of hydrates of borates and canavesite. The borosilicates showed an intermediate behaviour, as in the case of manganaxinite and dravite (Table 3, entries 16 and 12). Note that **8** was not obtained with the simple boron compounds used as references (Table 3, entries 20–21). Since the synthesis of **8** from formamide invariably requires the generation of hydrogen cyanide (HCN) (Yamada et al. 1978), it is reasonable to suggest that hydrates of borates, borocarbonates and simple boron derivatives were not efficient catalysts for this transformation. The generation of HCN during the condensation of formamide was confirmed by the detection of the HCN-tetramer, diaminomaleodinitrile (DAMN), in the presence of dravite and vonsenite (note [l], Table 3) (Orgel 2004). The selectivity in the

Table 4 Quantitative profile of compounds **9**–**17** produced by the condensation of formamide and mineral borates[a]

Entry	catalyst	Yield [mg g^{-1}][b]								
		(**9**)	(**10**)	(**11**)	(**12**)	(**13**)	(**14**)	(**15**)	(**16**)	(**17**)
1	Borax[c]	n.d.	0.002	n.d.	0.008	0.002	n.d.	0.02	n.d.	0.002
2	Colemanite[c]	0.18	0.001	0.65	0.02	n.d.	n.d.	n.d.	0.05	0.001
3	Hydroboracite[c]	n.d.	0.001	n.d.	0.007	0.001	n.d.	0.030	<0.001	<0.001
4	Kernite[c]	n.d.	<0.001	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5	Kurnakovite[c]	0.01	0.002	n.d.	<0.001	n.d.	n.d.	0.03	0.027	0.022
6	Ulexite[c]	0.09	0.004	0.99	0.003	n.d.	n.d.	n.d.	0.003	n.d.
7	Chambersite[d, i]	n.d.	0.004	0.19	0.005	n.d.	n.d.	0.006	0.09	<0.001
8	Hambergite[d]	0.16	n.d.	1.82	0.055	n.d.	n.d.	n.d.	n.d.	n.d.
9	Ludwigite[d, h]	n.d.	0.002	0.61	0.005	n.d.	n.d.	n.d.	n.d.	<0.001
10	Rhodizite[d]	0.005	0.002	1.26	0.004	n.d.	n.d.	0.018	n.d.	n.d.
11	Vonsenite[d]	0.21	0.073	3.95	n.d.	n.d.	n.d.	n.d.	<0.01	n.d.
12	Dravite[e, i]	n.d.	0.173	n.d.	0.171	n.d.	1.16	0.059	n.d.	n.d.
13	Dumortierite[e]	n.d.	0.003	0.037	0.011	n.d.	0.033	<0.001	n.d.	n.d.
14	Elbaite[e]	n.d.	0.015	0.008	0.019	n.d.	0.054	<0.001	n.d.	0.002
15	Kornerupine[e]	n.d.	0.003	0.32	0.012	n.d.	0.045	<0.001	0.006	0.002
16	axinite-(Mn)[e]	n.d.	0.003	0.023	0.012	n.d.	0.045	0.004	0.004	n.d.
17	Schorl[e]	0.05	0.007	0.35	0.030	n.d.	0.020	0.006	0.005	n.d.
18	Canavesite[f]	n.d.	0.130	0.21	0.002	0.005	n.d.	0.006	0.143	n.d.
19	Painite[g, l]	n.d.	0.007	0.45	0.016	n.d.	0.026	0.001	n.d.	0.001
20	Boric anhydride	n.d.	0.003	1.10	0.012	0.001	n.d.	0.033	0.003	0.003
21	Na perborate	n.d.	0.002	5.47	0.001	n.d.	n.d.	n.d.	n.d.	0.011
22	None	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] Products were identified by comparison of their retention time and mass-spectra with those of authentic samples. [b] Quantitative evaluation was performed by gas-chromatography/mass-spectrometry analysis (GC) as described in the experimental section. [c] Group A. [d] Group B. [e] Group C. [f] Group D. [g] Group E. [h] Traces of succinic acid. [i] Traces of parabanic acid and malonic acid. [l] A significative amount of malonic acid was also detected (0.01 mg/gr)

synthesis of compounds **5**–**6** requires the following discussion. Compound **5** was produced by a number of borate minerals higher than that available for **6**, hydrates of borates (Table 3, entries 3 and 5), anhydrous borates (Table 3, entries 7 and 9–10), borocarbonate (Table 3, entry 18) and borosilicates (Table 3, entry 12) being the best catalysts. The reaction pathway for the formation of **5** from formamide is similar to that for **6** (Yamada et al. 1978), the only difference being the selectivity in the addition/elimination process of ammonia as nucleophile on the substituted 5,6-dihydro pyrimidinone intermediate (that is C-2 versus C-4 addition/elimination process. For the numbering of the pyrimidinone ring see Scheme 1). The prevalence of **5** with mineral borates highlights the role of the mineral surface in the regioselectivity of the condensation (Botta et al. 1997). Uracil, a product of elimination of ammonia from both **5** and **6**, was detectable with borosilicates (Table 3, entries 12–17), multiple oxide (Table 3, entry 19), colemanite, chambersite and vonsenite (Table 3, entries 2, 7 and 11). Hydroxypyrimidines **2**–**3** were obtained under various experimental conditions, dravite being the best catalyst (Table 3, entry 12).

Qualitative structure activity relationships (SAR) for each Dana's group were also defined applying the Nickel-Strunz borate minerals classification rules (Strunz and Nickel 2001). As examples, in the borosilicates, the member of the $[Si_6O_{18}]^{12-}$ six membered single ring sub-class, dravite was more reactive than sorosilicates (kornerupine) and neosilicates with BO_3 triangles and B[4] and Be[4] tetrahedra [durmortierite and axinite-(Mn)]. Similarly, the hydrates of borates classified as triborates (colemanite and kurnakovite) were more reactive than tetraborates and pentaborates. Monoborates were the more reactive anhydrous borates.

Synthesis of Biogenic Carboxylic Acids and Amino Acid Derivatives

In addition to compounds **2**–**8**, carbodiimide, carboxylic acids and amino acid derivatives were synthesized (compounds **9**–**17**, Scheme 2 and Table 4). Biogenic carboxylic acids lactic acid **10** and pyruvic acid **14** are intermediates in the reductive version of the citric acid cycle. This cycle was suggested by Smith and Morowitz (2004) as a primordial autocatalytic reaction pathway for the reductive carbon assimilation of carbon oxides in a primordial metabolism (Morowitz et al. 2000). The variant of the conventional (oxidative) citric acid cycle is known to be a metabolic process in some bacteria (Holms 1987). Examples of prebiotic synthesis of intermediates of the reductive version of the citric acid cycle were previously reported for the formation in small yield of pyruvic acid **14** from carbon monoxide (CO) and iron sulfide and alkanethiols (Cody et al. 2000), and the formation of acetic acid form CO in with iron-nickel and sulfides (Huber and Wächtershäuser 1997). Moreover, traces of ketoglutaric acid were obtained from succinate in the presence of zinc sulfide under photochemical conditions (Zhang and Martin 2006). Glycolic acid **13** is involved in the photosynthetic oxidation of glycolate to glyoxylate (Smith et al. 1997), while glyoxylic acid **15** is an intermediate (in the salt form) in the glyoxylate cycle in bacteria, fungi and plants (Escher and Widmer 1997). Compounds **10** and **13**–**15** can be produced, in accordance to Eschenmoser suggestions (Eschenmoser 2007), through a common reaction pathway involving a reactive HCN-dimer and/or DAMN. The hydrolysis of the nitrile moiety in DAMN affords hydroxyoxaloacetic acid tautomers (2,3-dihydroxymaleic, 2,3-dihydroxyfumaric, and 2-hydroxyoxaloacetic acid, respectively) that are easily converted to oxaloacetic acid (not shown) and its decarboxylation product **14** *via* a stepwise addition of over two electrons and two protons (Eschenmoser and Loewenthal 1992; Koerber et al. 2005). Further reduction of **14** yields **10**. In a similar way, **15** can be formed by hydrolysis of the HCN-dimer, and **13** by a second two electrons-two protons reductive step of the formyl moiety in **15**.

The presence of the dimer of lactic acid, compound **11**, is also of interest. In fact, even though **11** is not an intermediate of present day metabolism, its formation suggests the possibility of spontaneous dimerization processes during the condensation of formamide, possibly catalyzed by carbodiimide **9**. Compound **9** is a well-known condensing agent for the synthesis of peptides and oligonucleotides starting from deactivated monomers and, in principle, it may play a role in abiotic polymerization processes (Slebocka-Tilk et al. 2002). As previously reported, **9** was produced by elimination of H_2O from the urea generated in situ during formamide condensation (Costanzo et al. 2007). This hypothesis was confirmed by the efficient synthesis of **11** treating **10** in the presence of 1-ethyl-3(3-dimethylamino) carbodiimide (EDC), a commercially available and relatively stable derivative of carbodiimide. The synthesis of *N*-formylglycine **16** was previously reported in the presence of mineral phosphates to occur by a Strecker condensation between HCN, formaldehyde

and ammonia (Miller 1953), followed by a *N*-formylation step with excess formamide (Aizpurua and Palomo 1983). *N*-formyl alanine **17** was not previously obtained from formamide. In this latter case, a Strecker condensation involving acetamide can be suggested, even though the methylation of **16** with formaldehyde, or the amination of pyruvic acid with ammonia, cannot be completely ruled out. An example of the alkylating properties of formaldehyde in the presence of minerals was reported by Kulkarni et al. (2008). The formation of amino acids from their ketone precursors by reductive amination or transamination are well documented reactions (Doctor and Orò 1967; Huber and Wächtershäuser 2003). Moreover, hydrolysis of DAMN under vigorous conditions has been reported to afford mainly glycine in the presence of small amounts of alanine and aspartic acid (Sanchez et al. 1967; Ferris et al. 1978).

As for the selectivity of the reactions, all mineral borates behaved as catalysts for the synthesis of compounds **10**–**12**. A major selectivity was observed for carboxylic acids **13**–**15**, as exemplified by pyruvic acid **14** that was synthesized only in the presence of borosilicates (Table 4, entries 12–17) and painite (Table 4, entry 19). Borosilicates were also the best catalysts for the preparation of glyoxylic acid **15**. Hydrates of borates (with the only exception of borax and kernite) (Table 4, entries 1–6) and one of the anhydrous borate group, chambersite (Table 4, entry 7), were also catalysts for the synthesis of both *N*-formylglycine **16** and *N*-formyl alanine **17**. Several borosilicates (kornerupine, axinite-(Mn) and schorl) (Table 4, entries 15–17) and one mineral of the anhydrous borate group (vonsenite) (Table 4, entry 11) selectively catalyzed the synthesis of **16**, while **17** was detected in the presence of ludwigite, elbaite and painite (Table 4, entries 9, 14 and 19).

Conclusions

In the context of the origin of nucleic acids, dry-wet cycles were suggested as possible mechanisms for the solution of the "water paradox", the thermodynamic conundrum impairing the likelihood of polymerizations to occur and give rise to stable polymers. Water is the only environment in which RNA and DNA conceivably evolved to their-present function and its chemistry has necessarily entered the prebiotic scenario at some point during the evolution of chemical pre-genetic complexity. In addition, dry-wet cycles also offer plausible frames for the prebiotic chemistry of formamide and mineral borates. Formamide, the product of hydrolysis of HCN, is easily concentrated by evaporation of water, thus accumulating in dry environments. The possible hydrolysis of formamide to ammonium formate is not deleterious in this context, being ammonium formate a known precursor for the synthesis of purine nucleobases (Hill and Orgel 2002). Borate minerals are often found on Earth as evaporites, the mineral deposits produced during the evaporation of water. Thus, in principle, it appears that in a coherent set of conditions, essentially consisting of alternating wet-dry cycles, the possible concentration of formamide and the formation of deposits of boron minerals in the form of evaporites (Grew et al. 2011), were all contemporaneously possible. Hence, the relevance of the question of whether formamide and mineral borates are effective reagents in the processes leading to the synthesis of compounds necessary for the origin of life. The data reported show that formamide oligomerizes in the presence of borate minerals yielding, at the same time, nucleobases and biogenic carboxylic acids. The synthesis of these compounds entails common precursors as HCN-oligomers. Noteworthy, different mineral studied were able

to catalyze the contemporary synthesis of both classes of biomolecules. The very fact that a one-pot, one-catalyst reaction affords the building blocks of both genetic and metabolic apparatuses highlights the interest of formamide-borates chemistry. The presence of amino acid derivatives, and the known catalytic effects of borates on prebiotic synthesis of ribose, further increase the interest of this experimental model.

Acknowledgement UE Cost Action CMO703 System Chemistry and the project ASI/INAF/015/07/0 are acknowledged.

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