Reports

Orgueil Meteorite: Organic Nitrogen Contents

Abstract. *Purines, amino derivatives of* sym-*triazine, and substituted guanidines isolated from the Orgueil meteorite were identified by chromatographic, spectroscopic, and other techniques. The presence of large amounts of* sym-*triazine derivatives is of particular interest, because these compounds have no known biochemical significance.*

In 1961 Calvin (*1*) and Briggs (*2*) independently reported spectroscopic evidence of purine and pyrimidine bases in water extracts of carbonaceous chondrites. Oró (*3*) demurred, suggesting that the observed spectroscopic features were due to an impurity from the ion-exchange resin. Kaplan *et al.* (*4*) found no conclusive evidence from ultraviolet absorption data of the presence of these bases in meteorites. In order to resolve this question, I attempted to isolate these compounds without using ion-exchange resins.

Sixteen grams of finely powdered Orgueil meteorite (*5*) which had already been extracted with water, ether, cyclohexane, chloroform, benzene, acetone, methanol, isopropanol, and butanol were extracted by heating with 3*N* HCl in a sealed tube for 6 hours at 120°C. After evaporation under reduced pressure, the dark brown residue (7.692 g) was heated with acetic anhydride for 6 hours at 100°C; after filtration and removal of the solvent in a vacuum below 30°C, the reddish brown acetylated residue (187.32 mg) was extracted with butanol. The alcohol extract was washed with cold aqueous $NaHCO_3$ and cold water and dried, and the solvent was removed. The residue was fractionated by solubility methods in the following solvents: fraction A, 10.57 mg, chloroform; fraction B, 8.26 mg, hot chloroform (insoluble in cold chloroform); fraction C, 86.03 mg, anhydrous methanol (insoluble in hot chloroform); fraction D, 74.93 mg, 80 percent methanol (insoluble in anhydrous methanol).

The ultraviolet spectra of fractions A, B, and C indicated the presence of amino derivatives of aromatic-type compounds which are *p*H sensitive. Fraction A showed absorption maxima in 1*N* HCl at 238, 258, and 304 mμ; in alcohol at 255 to 256 mμ; and in 1*N* NaOH at 253 to 256 mμ (only a shoulder); fraction B, in 1*N* HCl at 241, 315, and 362 mμ; in alcohol at 252 mμ (shoulder); and in 1*N* NaOH at 251 to 253 mμ (shoulder); fraction C, in 1*N* HCl at 223, 250 to 252 (shoulder), and 343 to 346 mμ; in alcohol at 255 mμ; and in 1*N* NaOH at 252 to 256 mμ (shoulder).

The infrared spectra of fractions A, B, and C showed the presence of an amide carbonyl and a nitrogen-containing heteroaromatic ring (stretching vibrations 6.25 to 7.14 μ): in KBr, fraction A showed at 5.94, 6.21, and 6.58 μ; fraction B, at 5.94, 6.22, and 6.62 μ; and fraction C, at 5.94 (very weak), 6.17, and 6.52 μ.

Fraction A was purified on an alumina column [alumina, neutral, activity II on the Brockmann grade (*6*), 5 by 0.5 cm]. Fraction A1, eluted with 70 ml of $CHCl_3$, consisted of 0.20 mg of colorless oil; fraction A2, eluted with 100 ml of $CHCl_3$, of 2.38 mg of pale yellow solid; and A3, eluted with 150 ml of a mixture of $CHCl_3$ and CH_3OH at 19:1, of 2.09 mg of yellow solid. Judged by the infrared and ultraviolet spectra, fraction A2 seemed to be a mixture, mainly of diacetylmelamine (*7*) and small amounts of other acetyl derivatives of nitrogen-containing heteroaromatic compounds; its infrared spectrum showed absorption peaks in KBr at 3.05 to 3.15, 3.17, 5.77 (weak), 5.93, 6.19 to 6.20, and 6.62 μ; and its ultraviolet spectrum showed in 1*N* HCl at 234 to 236 and 255 to 256 mμ (shoulder). Diacetylmelamine showed in KBr at 3.05 to 3.12, 3.16, 5.93, 6.19, 6.36, and 6.62 μ; the ultraviolet spectrum showed in 1*N* HCl at 236 mμ.

Fraction A2 was hydrolyzed with 5 percent HCl for 18 hours at 30°C. Solvent removal in a vacuum below 30°C left 1.74 mg of the hydrochloride of the hydrolyzed product. Its yellow picrate, which decomposed between 297° and 310°C, yielded 0.63 mg of free base which was compared by thin-layer chromatography with 38 authentic purine, pyrimidine, and *sym*-triazine compounds; chromatography was carried out on a silica-gel G plate, a mixture of butanol, water, and acetic acid (84:15:1) being used as the developer. Of seven spots detected under ultraviolet light four were identified as melamine (R_F 0.26), ammeline (R_F 0.21), adenine (R_F 0.75), and guanine (R_F 0.62).

Fraction A3 was purified by the same procedure. The free base (0.402 mg), after purification through the picrate, was examined by thin-layer chromatography. Three compounds identified were melamine, ammeline (*8*), and guanine (Table 1); five other spots remained unidentified.

Although fractions B and C have not yet been examined in detail, their spectra and behavior showed the presence of a nitrogen-containing heteroaromatic compound and an aliphatic amine. The infrared spectrum of fraction C indicated a very weak band in the region of amide carbonyl stretching absorption; this suggested incomplete acetylation under the conditions used, or decomposition of the acetylated product by alcohol or water. These compounds might be similar to uracil: hydroxypurines with two or more hydroxyl groups, ammelide, and some sort of guanidine derivative.

The infrared spectrum and elemental analysis indicated that inorganic compounds were present in fraction D, 70 mg of which was further fractionated by solution in water and continuous extraction with butanol for 36 hours. The alcohol extract was distilled under reduced pressure, leaving 4.75 mg of pale brown residue. Treatment of the residue with 3 percent HCl for 10 hours at room temperature yielded 1.48 mg of amorphous solid hydrochloride (fraction D1). The infrared spectra of the hydrochloride and its free base showed that guanidine derivatives were present; the spectrum of the hydrochloride showed features at 2.92 to 3.03, 6.06, 6.20, and 6.51 μ (shoulder), and thus strongly resembled the spectra of *N,N-*

and *N'*-disubstituted guanidine hydrochlorides such as *N,N*-diethyl-*N'*-methyl guanidine hydrochloride.

In connection with the characterization of fraction D1, the infrared spectra of 26 hydrochlorides of primary and secondary amines and guanidine were measured. Further, the reported infrared spectra of over 100 amine hydrochlorides were compared with the spectra of the fractions from the meteoritic sample. The primary amino group gives rise to NH-stretching bands in the region of 2.86 to 3.03 μ, and the NH-stretching bands of its ammonium ion occur above about 3.08 μ (*9, 10*), whereas the NH-stretching bands of the guanidinium ion appear in the free amino region rather than in the ammonium region (*10, 11*). This suggests that the positive charge is localized mainly on the central carbon atom rather than distributed over the entire guanidinium group. Generally, mono- (*N*R– and disubstituted (*N*R–, *N'*R–) guanidine hydrochlorides (R being the alkyl or alicyclic group) give two bands between 5.92 and 6.39 μ because of the NH– deformation vibrations, while unsubstituted guanidine hydrochlorides (guanidine itself) and trisubstituted ones (*N*R–, *N'*R'–, *N''*R–) show only one band in the deformation region. The positive Sakaguchi reaction (*12*) indicates that monosubstituted guanidine groups also are present in this fraction. No ultraviolet absorption peak was detected in this fraction; there was a very weak shoulder in the region of 275 to 285 mμ. From these data it is deduced that mono- and disubstituted guanidines were present in fraction D1.

Although thin-layer chromatography of fraction D1 gave six spots which have not yet been identified, neither guanidine itself nor its simpler alkyl-substituted derivatives were detected on the chromatogram. Presumably fraction D1 consists of guanidine derivatives of iminazolidine, iminazoline, and iminazole. We know that acid hydrolysis of adenine (*13*) or guanine (*14*) leads to formation of iminazole derivatives under mild conditions. Conceivably, the guanidine derivatives found may be degradation products of nucleic acids or other compounds.

In marked contrast with the HCl-extract, the aqueous and organic-solvent extracts of the meteorite seemed to contain no basic nitrogen compounds except some sorts of amino acid. Their infrared spectra showed no absorptions due to either an amide carbonyl or a nitrogen-containing heteroaromatic ring, and the ultraviolet spectra indicated the absence of amino derivatives of aromatic-type compounds which are *p*H sensitive.

Table 1. Identified compounds in the Orgueil meteorite (μg/g, dry wt).

Compound	Fraction A2	Fraction A3
Melamine	20 ± 5	15 ± 5
Ammeline	13 ± 5	15 ± 5
Adenine	15 ± 5	
Guanine	11 ± 5	9 ± 5

The amount of nitrogen compounds isolated from the 16 g of Orgueil meteorite is far too great (1 to 2 mg) to reflect contamination from solvents and reagents (*15*). Although the solvents used contained 0.0 to 0.5 mg residue per 100 g of solvent, there were no extraneous absorption bands in their infrared and ultraviolet spectra, and, as mentioned earlier, these extracts contained no basic nitrogen compounds. As a test for contamination with biogenic material, the samples were examined for optical activity (*16*). No optical rotation was detected. While one cannot rule out a slight amount of terrestrial contamination in a century-old meteorite, the nature and amounts of nitrogen compound found strongly oppose contamination as a significant source.

What bearing do these results have on the purported evidence of life in meteorites? First, the presence of purines has now been unambiguously confirmed. Second, there is the problem of accounting for the origin of the melamine and ammeline; neither they nor other compounds with the *sym*-triazine ring system have any known biological significance. Either they were produced by an extraterrestrial life form whose biochemical pathways differ from those of terrestrial life, or they were made by abiotic processes. The former alternative cannot be excluded a priori, but it opposes all recent arguments for a biological origin of the meteoritic organic compounds. These arguments have cited the similarities between meteoritic organic matter and terrestrial biogenic materials as evidence of extraterrestrial life with a biochemistry closely duplicating that of terrestrial life. It is somewhat puzzling that a biochemistry conceded to differ from our own in one major respect should resemble it so closely in all others.

There seems to be no problem in accounting for the *sym*-triazine derivatives by abiotic processes (*17*). Such compounds can, in principle, be synthesized from hydrogen cyanide and ammonia or from a simple organic molecule under the conditions thought to have prevailed in the early solar system. Melamine can be prepared by chemical synthesis from hydrogen cyanide and ammonia, guanidine or biguanide, urea, carbon monoxide and ammonia, and many other substances. Although the mechanism of melamine formation from the starting materials mentioned has not yet been worked out in detail, the reaction may proceed through an active intermediate such as cyanamide. Mechanisms for the formation of adenine from hydrogen cyanide and ammonia under primitive earth conditions have been suggested by Calvin (*1*) and Oró (*18*), and several successful syntheses from hydrogen cyanide, ammonia, and other simple compounds have been reported (*18, 19*). Guanine was also formed (*20*) by thermal polymerization of amino acids.

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Transitional Ordovician Bivalve with Both Monoplacophoran and Lucinacean Affinities

Abstract. *The rare and problematic Ordovician molluscan genus* Babinka *is a probable evolutionary link between the bivalve superfamily Lucinacea and some monoplacophora-like ancestral mollusc.* Babinka *provides the first direct evidence of a transition between the class Bivalvia and more primitive molluscan ancestors.*

The recognition of the significance of multiple muscle scars in fossil monoplacophorans and the subsequent dramatic discovery of the recent monoplacophoran *Neopilina* have aroused interest in all early fossil molluscs with multiple muscle scars. One such fossil which has attracted wide attention is the rare Ordovician bivalve genus *Babinka,* which was first described by Barrande in 1881 (*1*). *Babinka* is known only from rocks of lowest Middle Ordovician (Llanvirn) age in the vicinity of Prague, Czechoslovakia and is among the first bivalves to appear in the fossil record. Barrande's original illustrations show a peculiar series of radial muscle scars in the dorsal region, and this muscle pattern led Vokes (*2*) to suggest that *Babinka* might be an evolutionary transition between the Bivalvia and some metameric ancestral mollusc. Vokes's proposal has been widely accepted by students of molluscan phylogeny (*3*).

A further study of all available specimens of *Babinka* has shown that the pattern of the muscle scars and the general morphology are more complex than had been suspected previously (*4*). *Babinka* has normal bivalve adductor and pallial muscles, but is unusual in having eight pairs of pedal muscles, instead of the normal bivalve pattern of two to five pairs. In addition, *Babinka* has a unique linear series of very small muscle scars below the third through seventh pairs of pedal muscle scars. These smaller scars probably represent the sites of gill muscle attachment. The pattern of pedal and gill muscle scars in *Babinka* is almost identical to the pattern in recent *Neopilina* and in some early fossil Monoplacophora (Fig. 1). These close similarities support Vokes's suggestion that the muscle pattern in *Babinka* is an inheritance from a monoplacophora-like ancestral mollusc. In all features except the pedal and gill muscle scars, *Babinka* is a typical isomyarian bivalve.

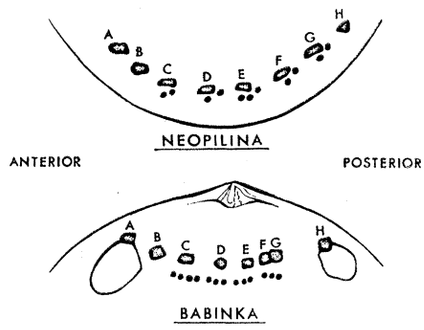

Fig. 1. Comparison of the patterns of the pedal and gill muscle scars in the Ordovician bivalve *Babinka* and the recent monoplacophoran *Neopilina.* The muscle scars labeled *A* through *H* are the attachment sites of the eight pairs of pedal muscles. The small solid dots below the pedal scars show diagrammatically the positions of the attachment scars of the gill muscles. In both *Babinka* and *Neopilina* the gill scars are associated with pedal scars *C* through *G*.

Babinka is both morphologically and chronologically an ideal ancestor for the large bivalve superfamily Lucinacea, which appears abruptly in the fossil record in Middle Silurian deposits. Among the morphological features of *Babinka* that are indicative of lucinoid affinities are the characteristic shape, elongate anterior adductor scar, nonsinuate pallial line, and typical lucinoid hinge, dentition, and ligament.

Functional morphological studies of recent Lucinacea by Allen (*5*) have shown that all members of the group share unusual adaptations for life as deeply buried suspension feeders. Unlike most deeply buried bivalves, lucinoids do not have posterior siphons for channeling nutrient-laden water into the mantle cavity. Instead, all Lucinacea have the peculiar ability to use the cylindrical foot for construction of a mucous-lined, anterior inhalant tube to the sediment surface. This habit is reflected in lucinoid shell morphology, for the characteristic anteriorly expanded shape and elongate anterior adductor muscle provide ciliary sorting surfaces for incoming food particles. Morphologic comparisons of *Babinka* and recent lucinoids suggest that *Babinka* was also an infaunal suspension feeder which used the foot to maintain an anterior inhalant opening to the surface of the sediment. The lucinoid habit of feeding through an anterior inhalant tube is apparently a very early bivalve specialization for an infaunal, suspension-feeding mode of life, for lucinoids appear in the fossil record long before the first typical siphonate bivalves. Such forms first occur in the Carboniferous, and do not become really abundant until Mesozoic time.

Babinka provides the first direct evidence of a transitional evolutionary link between the Bivalvia and some more primitive molluscan ancestor (Fig. 2). The phylogenetic position of *Babinka* indicates that the Lucinacea arose directly from monoplacophora-like ancestral molluscs. There is no strong zoologic or paleontologic evidence to suggest that the Lucinacea have given rise to other major groups of the Bivalvia, with the probable exception of the Leptonacea. This raises the possibility that the Bivalvia had a "polyphyletic" origin from less specialized ancestral forms. The early origin and separate evolutionary development of lucinacean bivalves suggests that the group should be assigned to a separate

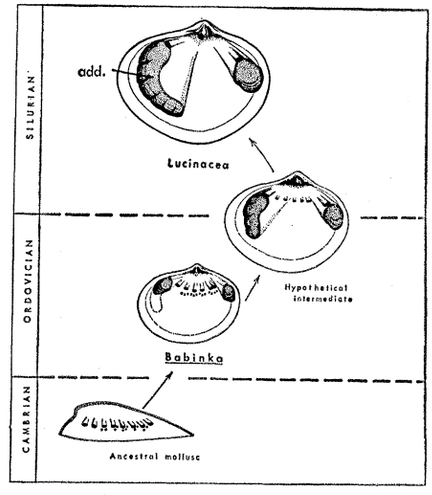

Fig. 2. Evolutionary relations of *Babinka.* The genus represents a transition between a monoplacophora-like ancestral mollusc and the large Silurian to recent bivalve superfamily Lucinacea. In the Lucinacea the pedal and gill muscles have been reduced while the anterior adductor (*add.*) muscle has expanded in size.