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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ISOLATION OF URIC ACID FROM SOIL FOR ISOTOPIC DETERMINATION

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SUMMARY

Uric acid was extracted from soil into potassium phosphate solution and isolated from other components by high-performance liquid chromatography, then the isolated fraction was subjected to nitrogen isotope analysis. Studies of the adsorption of uric acid on clay indicated no significant isotope fractionation during the exchange reaction; hence the isotope ratio found in the extract was the same as that in the soil. An increase in soil ammonia content followed the decomposition of uric acid. No uric acid isotopic preference by uric acid bacteria during the decomposition was found. The fractionation at the time of ammonia evaporation was mainly responsible for the high nitrogen isotope ratio in bird rookeries. An extremely high ratio for soil ammonia was found in a simulation experiment. This phenomenon may be mimicked in nature.

INTRODUCTION

It has long been known that the plant community in a sea-bird rookery is very different from that outside it. Indeed, the community would not exist if the birds were absent. The difference is especially apparent in the polar regions, where the plant communities around a breeding area often form a luxuriant, closed community, in contrast with surrounding barren areas[1–3]. Although all the reasons why a bird rookery affords such a peculiar ecosystem may not be straightforward to elucidate, the deposition of manure, feathers, eggs, food remains and bird bodies and the resulting large input of nutrients to the rookery must be a major cause of the establishment of the ornithocoprophilous plant community. In fact, annual inputs of nitrogen, phosphorus and potassium from bird excreta alone to a Black-tailed Gull rookery were found to be 53, 45 and 21 g/m^2, respectively, which are much greater than those to cultivated fields in some European countries, Korea and Japan[4], where the most fertilizer-intensive agriculture in the world is currently undertaken[5]. Heavy plant growth in contrast with generally scarce vegetation outside the rookery results from such a large input of nutrients[6,7].

As most of the nitrogen in bird excreta is in the form of uric acid (often as

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high as 70–90%), which is not directly utilized by higher plants, its transformation by uric acid bacteria must occur at the beginning of the nitrogen cycle in excreta deposited on rookery soil. The degradation of uric acid to ammonia and its evaporation must play an important role in the nitrogen cycle in bird rookeries. However, very little is known about the rate of faecal production or nitrogen transformation[8,9], and the knowledge of their quantitative significance in natural ecosystems remains minimal[10].

As isotopic studies of the nitrogenous substances in an ecosystem often elucidate their biogeochemical nitrogen dynamics[11,12], we considered it desirable to isolate uric acid from rookery soil and to determine its isotopic composition. As we failed to find a literature method that could be used for this purpose, we chose to develop a high-performance liquid chromatography (HPLC) technique to isolate and prepare uric acid from soil for later isotope determination.

The preparation of a compound for later isotopic analysis generally has a unique primary requirement: the need for complete isolation and symmetrical collection[13] of a few milligrams of the compound with no introduction of exogenous isotopes. Milligram amounts of the pure compound are necessary because current isotopic measurements commonly require this amount to give reliable results. Complete isolation was required not only for the elimination of possible contaminants but also to achieve the symmetrical collection that is essential in order to effect an accurate isotope measurement, as isotopic fractionation occurs during chromatographic procedures[14].

The above requirements also apply to uric acid in soil and are an additional prerequisite for the present study. The HPLC determination of uric acid in soil and a discussion of its application to the study of rookery soils to elucidate their unique biogeochemical features is given in an accompanying paper[15]. In this paper we present an HPLC method for the isolation of uric acid from soil for later isotopic determination. Some findings of biogeochemical interest are also given to illustrate a potential utility of the method.

EXPERIMENTAL

Chemicals and clay

Water was distilled from demineralized water in an all-glass Yamato Model WAG-24 Auto Still (Yamato Kagaku, Tokyo, Japan). Uric acid and potassium phosphate monobasic salt were of guaranteed reagent grade from Wako (Osaka, Japan). Potassium chloride used for the extraction of soil ammonia was also of guaranteed reagent grade from Wako and was heated in a muffle furnace at 500°C for 2 h before dissolving it in water to give a 2 *M* KCl extractant solution. A standard clay mineral (Swy-1 Na^+-montmorillonite from Crook County, WY, U.S.A.) was obtained from the Source Clay Mineral Repository (Department of Geology, University of Missouri, Columbia, MO, U.S.A.) and was used without further treatment. The cation-exchange capacity (CEC) of the clay was 1.01 mequiv./g dry clay[16].

Bird excreta

Excreta of the Black-tailed Gull (*Larus crassirostris*) was collected on June 22, 1983, at Kabushima rookery (Hachinohe, Aomori Prefecture, Japan). Immediately

after collection, it was weighed, a known amount of ethanol was added and the container was tightly sealed. After arrival at laboratory, the container was opened and the contents were vacuum-dried. The content of volatiles in the excreta was 68% and 29% of the dried matter was uric acid.

Preparation of uric acid-containing soil

Surface soil (0–5 cm) from the Kabushima Black-tailed Gull rookery was sampled on June 20, 1983. It was about 1 week before the beginning of the departure of the gulls, and their density was approximately two adults and one fledgling per square metre[17]. The body size of a fledgling was in general comparable to that of an adult gull.

A 20 × 20 cm section of surface soil (0-5 cm depth) was sampled, taking as much care as possible to keep the soil structure of the section intact. It was then placed in a plastic container and kept at 4°C for 2 weeks in order for uric acid bacteria to consume indigenous uric acid[8]. After the incubation, two 52-mm diameter cores were made using a plastic core sampler and placed separately, right side up, at the bottom of a plastic cylinder of 52 mm I.D. Uric acid was then sprinkled on the top of one soil core. The amount of uric acid was 3.00 g per 100 g of wet soil and roughly equalled the annual input of uric acid to the corresponding surface area of soil in the rookery. No uric acid was added to the other core. When the uric acid covered the surface of the core, 18.0 ml of water per 100 g of wet soil was sprinkled to the soil. The ratio of the amount of added water to the amount of uric acid was about the same as that found in the excreta of Black-tailed Gulls. The core without the addition of uric acid also received water in the same proportion to the amount of wet soil. A lid was then placed at the top of the cylinder. All the above procedures were carried out in a cold room kept at 4°C.

The cylinders were moved to a 20°C constant-temperature room and incubated for 10 days with a 100 ml/min flow of water-saturated, ammonia-free air through the gas phase of the cylinders, the volume of which was about 100 ml. The air that passed through the cylinders was bubbled into a 1 *M* H_2SO_4 trap for evolved ammonia. After the incubation, the soil was vacuum-dried. A series of H_2SO_4 traps were placed between the cylinder and the dryer during the drying procedure so that all the ammonia evaporated from the soil would be trapped. The dried soil was filtered through a 2-mm stainless-steel sieve, homogenized to pass a 0.5-mm sieve and subjected to the extraction of uric acid. Although the extraction procedure was modified to handle larger amounts of the soil for performing isotopic analysis, it was basically the same as that given in the accompanying paper[15]. The same procedure was also employed for the extraction of uric acid from bird excreta.

Chromatography

Potassium phosphate solution for use in the HPLC mobile phase was pressure-filtered through a 0.4-μm microporous polycarbonate membrane (Nuclepore, Pleasanton, CA, U.S.A.).

Either one of two sets of chromatographic apparatus was used. One set was used for both analytical and preparative purposes, and consisted of an Erma ERC-3310 degasser (Erma Optical Works, Tokyo, Japan), a Waters Model 6000A dual-piston reciprocating pump (Waters Assoc., Milford, MA, U.S.A.), a Waters Model

U6K universal injector equipped with a 10-ml injection loop and a Waters Model 440 absorbance detector. The detector monitored at both 280 and 254 nm; the former was used to follow the elution of uric acid and the latter for detecting contaminants from soil. Chromatograms were recorded on a Pantos Unicorder U-225MS32 two-pen recorder (Nippon Denshi Kagaku, Kyoto, Japan). The entire apparatus was operated at 19–23°C.

The other apparatus, which was used mostly for preparative purposes, consisted of a Jasco DG-3510 degasser (Japan Spectroscopic, Tokyo, Japan), a Jasco TRI Rotar-V HPLC pump, a Jasco Model VL-614 variable-loop injector equipped with a 15-ml injection loop and a Jasco Uvidec-100-VI UV spectrophotometer. Two different quartz cells were used for the detection of uric acid: a standard 10-mm light-path cell for analytical purposes and a 0.1-mm light-path cell for the accurate analysis of highly concentrated solutions and for preparative purposes. The spectrophotometer was set to monitor at 286 nm. Chromatograms were recorded by a Hitachi 056-3001 two-pen multi-range recorder (Hitachi, Tokyo, Japan). The entire chromatographic system other than the columns was operated at ambient temperature. The column temperature was monitored and set at 34°C by a Thermoeye SI-1 temperature regulator (Iuchi, Osaka, Japan). The deviation of the column temperature from 34°C was less than 0.4°C.

The preparative column selected was a commercial 50 × 2.0 cm I.D. stainless-steel column (SUS 316) packed with polyhydroxyalkyl methacrylate gel (Shodex OHpak B-2004, Showa Denko, Tokyo, Japan). A pre-column (Shodex OHpak B-2000P) was used to protect the preparative column. The analytical column (Shodex OHpak B-804) was a commercial 50 × 0.8 cm I.D. stainless-steel column (SUS 316) packed with the same gel as the preparative column. A pre-column (Shodex OHpak B-800P) was used to protect the analytical column.

When the amount of uric acid was to be determined from a chromatogram, the peak heights were read by a Kontron MOPAM 02 semi-automatic image analyser (Kontron Messgeräte, Munich, F.R.G.), and the data were transferred to a Hewlett-Packard HP-85 personal computer that handled the data and printed out the amount of uric acid present in each sample.

Chemical analysis

An appropriate fraction of eluent was collected in a Kjeldahl flask and total uric acid in the fraction was measured spectrophotometrically with a Shimadzu UV-210A double-beam spectrophotometer (Shimadzu Seisakusho, Kyoto, Japan). Standard solutions of uric acid were used for the calibration. After the total uric acid had been determined, the fraction was frozen and lyophilized with a Labconco FDC-8 freeze-dryer (Labconco, Kansas City, MO, U.S.A.). Organic nitrogen in the lyophilized fraction was converted into ammonium by Kjeldahl digestion. The digestion was carried out for 4 h in the presence of a small amount of HgO–SeO–K_2SO_4 (3:1:4, w/w) as a catalyst[18]. The ammonium thus produced was steam-distilled and collected in a 0.125 *M* H_2SO_4 trap. The ammonium sulphate solution obtained was diluted to volume with water in a 100-ml volumetric flask. Prior to mass analysis, total nitrogen in the fraction was determined by using an aliquot of the diluted ammonium sulphate solution. The phenol–hypochlorite method[19] was employed for the determination, and the absorbance at 640 nm was measured with a Shimadzu

UV-210A double-beam spectrophotometer. Standard solutions of ammonium chloride were used for calibration.

Total organic carbon in an HPLC-isolated fraction was measured by combusting it as described elsewhere[20] to form carbon dioxide. The volume of the gas thus generated was measured manometrically, and converted to give the organic carbon content of the fraction.

Mass analysis

The ammonium solution was concentrated in an Ikeda VOD-4-2 vacuum drying oven (Ikeda Rika, Tokyo, Japan). It was then converted *in vacuo* to nitrogen gas with alkaline hypobromite. The nitrogen gas thus produced was purified by passing it through a CuO furnace with Pt wire heated at 700°C and through a Cu furnace heated at 400°C. Stable nitrogen isotope measurements were made with a Hitachi RMU-6R mass spectrometer with a dual inlet system and a double collector for ratiometry. The nitrogen isotope ratio was expressed as the per mill (‰) deviation from atmospheric nitrogen as defined by the following equation:

$$\delta^{15}\text{N (‰)} = \frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}} - (^{15}\text{N}/^{14}\text{N})_{\text{air}}}{(^{15}\text{N}/^{14}\text{N})_{\text{air}}} \cdot 1000$$

Two ammonium sulphate solutions with δ^{15}N values of -3.4 and 1.3‰ were used as standards. The standard deviation of the nitrogen isotope measurements was 0.2‰.

Isotopic analysis of ammonia nitrogen and total nitrogen

The procedure for the extraction of soil ammonia for later isotopic analysis was a modification of that of Bremner and Keeney[21] for the determination of soil ammonia content, and was carried out as follows. Approximately 5 g of soil were weighed into a 300-ml erlenmeyer flask, 100 ml of 2 *M* KCl solution were added and the flask was capped with a silicone stopper and shaken vigorously at room temperature for 1 h. After shaking, the contents were transferred into 50-ml centrifuge tubes and centrifuged at 3000 rpm for 10 min using a Kokusan H-103N table-top centrifuge (Kokusan Centrifuge, Tokyo, Japan). After centrifugation, the supernatant was pressure-filtered through a pre-heated (at 420°C for more than 5 h) Whatman glass-fibre filter (GF/C; diameter 4.7 cm; Whatman Maidstone, U.K.) and a 0.45-μm microporous filter of the same diameter (Type HA; Millipore, Bedford, MA, U.S.A.). The volume of the filtrate was measured, then the filtrate was subjected to steam distillation and the ammonia evolved was collected in a 0.125 *M* H_2SO_4 trap. When the steam distillation could not be carried out immediately, 1 ml of 0.025 *M* H_2SO_4 solution was added to the filtrate and it was stored at 4°C until ready for the distillation. Normally, the filtrate was kept in a cold room for no more than 20 h. The analytical procedure after the steam distillation was the same as that given above for the uric acid fraction.

The determination of the amount of total nitrogen and its isotopic analysis in soil and bird excreta was performed as follows. An appropriate amount of a sample was weighed and the organic nitrogen in it was converted into ammonium by the same Kjeldahl digestion as described earlier for lyophilized uric acid fractions; the subsequent procedure was the same as that for the uric acid fraction.

Adsorption of uric acid on clay

An aqueous solution of uric acid (25 mg/l) was prepared and 313.8, 157.0 and 78.3 mg of the clay were added to three 1-l portions of the solution, giving a series of three clay suspensions in which the acid present accounted for approximately 8, 16 and 32% of the dry weight of the clay present in the suspensions. The suspensions were then gently stirred at room temperature for 28 h in order to achieve nitrogen isotope equilibrium between the uric acid in the liquid phase and that adsorbed on the clay[22]. After the incubation, the suspensions were pressure-filtered through a pre-heated (at 420°C for more than 5 h) Whatman GF/C glass-fibre filter (diameter 4.7 cm) and a 0.4-μm microporous polycarbonate membrane of the same diameter (Nuclepore).

Most of the clay remained on the glass-fibre filter and a small fraction on the membrane filters; about 3 mg dry weight of clay was found on the membrane filters and it accounted for 1, 2 and 4% of the total amount of clay used for the 8, 16 and 32% experiments, respectively. The clay on the glass-fibre filter was subjected to Kjeldahl digestion together with the filter, and the ammonia thus generated was considered as to be from adsorbed uric acid.

The amount of uric acid in the filtrate was determined using the HPLC system equipped with the analytical column. After the determination, an appropriate portion of the filtrate was lyophilized in a Kjeldahl flask using a Labconco freeze-dryer and Kjeldahl-digested to convert uric acid nitrogen into ammonium. The amount of ammonia thus produced was measured spectroscopically, and the result agreed with that predicted by HPLC. This ammonia was regarded as being from uric acid in the liquid phase.

RESULTS AND DISCUSSION

Nitrogen from the preparative column

The packing material, polyhydroxyalkylmethacrylate gel, contains no apparent nitrogen and little nitrogen, if any, is expected to leave the column. However, its possible elution was still examined, as even a very small amount of exogenous nitrogen may alter the nitrogen isotope ratio of a uric acid fraction.

KH_2PO_4 solution (0.1 *M*) that was used both as the extractant and as the mobile phase was injected into the preparative column, and the fraction in which uric acid would elute was collected. The collected eluent showed no absorbance around 286 nm. The amount of organic nitrogen in the eluent was measured spectroscopically after digesting approximately 200 ml of the eluent, which was slightly more than the volume normally collected for a uric acid fraction. The organic nitrogen present in the digest amounted less than the detection limit of the method employed, which was about 3 μg N. As nitrogen isotopic determinations in this study were performed with more than a few milligrams of nitrogen, the baseline contamination of nitrogen accounted for no more than 0.1% of the total nitrogen collected.

The nitrogen content and the isotope ratio of the uric acid fraction also demonstrated the absence of exogenous nitrogen. Standard uric acid solution was injected and the same uric acid fraction was collected. The amount of uric acid was measured by the absorption at 286 nm, and the total organic nitrogen in the fraction was obtained after the Kjeldahl digestion. The nitrogen content of the uric acid in the

fraction was thus calculated to be 33.4 ± 0.5% (two analyses), the theoretical value being 33.33%. For the carbon content of the fraction, it has been reported elsewhere[20] that the uric acid fraction of Adelie Penguin excreta gave a calculated carbon content of 35.7 ± 0.3% (three analyses), the theoretical value being 35.72%. Further, the nitrogen isotope ratio of uric acid after the chromatography was the same as that before the chromatography. The nitrogen isotope ratio of the uric acid fraction was 15.8 ± 0.4‰ (six analyses) when the acid whose nitrogen isotope ratio was 16.2 ± 0.5‰ (four analyses) was chromatographed using the present HPLC system.

Nitrogen isotope fractionation during chromatography

Isotope fractionation during chromatography seems to have first been discovered for molecules that contain radioactive isotopes, and that of large molecules has been reviewed[14]. The fractionation is now known to occur to stable isotopes, and the recent literature on the subject includes carbon of CF_4 and sulphur of SF_6[23], carbon of CH_4 and CO_2 and oxygen of CO_2[24], and carbon of vanillin[25] during gas chromatography, nitrogen of aspartic acid during cation-exchange liquid chromatography[26], and carbon of uric acid during HPLC[20].

As the isotope fractionation during chromatography may make the isotope ratio of an isolated fraction different from that of the whole fraction, the extent of the nitrogen isotope fractionation must be known. Therefore, the chromatographic fractionation of nitrogen isotopes of uric acid during its isolation using the present HPLC system was examined. It was found that the uric acid eluted earlier under the present conditions was enriched in ^{15}N. This tendency coincided with our earlier experiences with carbon isotopes in uric acid that was fractionated under the same HPLC conditions[20].

The extent of the fractionation was as follows. The nitrogen isotope ratio of the first half of the uric acid peak had a value of 18.6‰ and that of the second half a value of 13.3‰, whereas the nitrogen isotope ratio of the whole peak was 15.9‰. Numerical analysis[13] of the fractionation showed that it is not large enough to necessitate particular precautions for completely quantitative recovery as far as symmetrical collection of the chromatographic peak is performed. This has also been demonstrated experimentally. When the middle 48.7% portion of a uric acid peak was collected, its nitrogen isotope ratio was 15.8‰, the difference of which from that of the whole peak (15.9‰) was within the experimental error. Throughout the present study, however, an effort was made to achieve a quantitative recovery of uric acid, and baseline collection of the uric acid fraction was always performed.

Examination of ammonia extraction method

In order to examine the extent of ammonia extraction, the procedure was repeated three times using the same uric acid-containing soil. The total amount of ammonia extracted by the three consecutive extractions was 12.7 mg N/g dry soil, and the first extract accounted for 98.0% of the total, the second for 1.3% and the third for 0.7%. After the steam distillation of the three extracts, none gave an ammonia content different from those obtained before the steam distillation. The nitrogen isotope ratio of the ammonia in the second extract was 1.8‰ lower than that in the first extract, while the third extract yielded a 8.7‰ lower ratio than the first. The considerably lower value for the third extract might reflect some change in the nature

of the extracted ammonia, and indicates an interesting course of investigation. For the present study, however, it should rather be emphasized that the overall value of the nitrogen isotope ratio of the soil ammonia in the three extracts is only 0.1‰ lower than that found in the first extract. Therefore, the ammonia content and the isotope ratio obtained from the first extract appeared sufficiently representative for the present purpose.

Nitrogen isotope fractionation during uric acid exchange reactions with clay

Nitrogen isotopic fractionation has been reported to occur during ammonium adsorption by clay colloids, and the isotopic equilibrium constant for the ammonium adsorption reaction was as high as 1.011 for Ca^{2+}-saturated clay colloids[22]. Therefore, the extent of the fractionation of nitrogen isotopes during the uric acid exchange reaction with soil needs be evaluated, as the present study was aimed at establishing an HPLC method to isolate uric acid from soil and to measure its nitrogen isotope ratio in order to elucidate nitrogen dynamics in rookery ecosystems.

The extent of possible bacterial growth and its consequent effect on the experiment were estimated by examining the balance of uric acid before and after the experiment. Uric acid present in the liquid fraction after reaching isotopic equilibrium was measured directly by the present HPLC system, and was 22.2, 22.1 and 20.9 mg for the 8, 16 and 32% experiments, respectively. The amount of uric acid adsorbed on the clay was measured spectroscopically after the Kjeldahl digestion of the clay on the glass-fibre filter. As 1 g of the clay contained 0.036 mg of indigenous, organic nitrogen[15], and as its nitrogen isotope ratio was −2.1‰, the organic nitrogen indigenous to the clay should alter neither the amount of adsorbed uric acid nor its nitrogen isotope ratio. After taking into account the acid on the clay that remained on the membrane filter, the total amount of adsorbed uric acid was calculated to be 3.9, 4.2 and 3.5 mg for the 8, 16 and 32% experiments, respectively. These uric acid contents found after the equilibrium experiment, therefore, amounted to 104, 105 and 98%, respectively, of the acid intially present in the clay suspension. It thus eliminates the possibility of bacterial interference with the equilibrium experiment.

Table I shows the results of the uric acid exchange experiment. The difference between the nitrogen isotope ratio of uric acid in the adsorbed fraction and that in the liquid fraction fluctuates, and averages 0.4‰ with a standard deviation of 0.5‰. The adsorbed fraction might slightly favour the heavy isotope; however, the difference is not very large in comparison with the standard deviation of itself and that of the nitrogen isotope measurements (0.2‰). Therefore, nitrogen isotope fractionation

TABLE I

NITROGEN ISOTOPE RATIOS (‰) OF URIC ACID IN A CLAY SUSPENSION

The nitrogen isotope ratio of uric acid used for the experiment was 15.9 ± 0.5‰ (standard deviation; 10 determinations).

Fraction	*Acid 8%*	*Acid 16%*	*Acid 32%*
Adsorbed fraction	15.4	16.5	16.3
Liquid fraction	15.5	15.6	15.8

during the uric acid exchange reaction with soil must be small, if any, and the isotope ratio found in the extract can be regarded as representative of the soil.

Uric acid metabolism and isotope ratio of nitrogenous fractions of soil

The water content of the soil used for making the uric acid-containing soil was 49.5 ± 1.6% (two analyses). After the water content had been measured, total organic nitrogen and its isotope ratio in the dried soil were measured, and found to be 27.1 ± 4.9 mg N/g dry soil (two analyses) and 20.8 ± 0.3‰ (two analyses), respectively. The amount of uric acid indigenous to the soil was obtained by sampling two separate cores from the soil section, and was 0.34 ± 0.34 mg N/g dry soil (two analyses). This uric acid must have come from bird excreta, and the large standard deviation may reflect a short-term heterogeneity of the mode of deposition of bird excreta and of the bacterial decomposition.

Three determinations indicated that the total nitrogen in the excreta collected in the rookery 2 days after the sampling of the soil was 173.7 ± 1.5 mg N/g dry excreta, of which 97.2 mg N/g dry excreta accounted for nitrogen from uric acid. The nitrogen isotope ratio of the whole excreta was 9.2 ± 0.4‰ (three analyses), whereas that of the uric acid alone was 9.1 ± 0.4‰ (four analyses). The purity of the isolated uric acid from the excreta was 101.5 ± 3.5% (two analyses).

As 30.0 mg of uric acid, or 10.0 mg equivalent of nitrogen, the nitrogen isotope ratio of which was 15.9 ± 0.5‰ (ten analyses), was added to every 1 g of wet soil, the amount of added nitrogen to the soil core in the form of uric acid was 19.8 mg N/g dry soil. Therefore, the combined amount of uric acid-nitrogen initially present on the surface of the core was 20.1 ± 0.3 mg N/g dry soil. And the overall nitrogen isotope ratio of the acid was 15.8 ± 0.5‰.

Table II shows nitrogen content and its isotopic ratio at the end of the incubation in different fractions of the soil. The amount of uric acid was 5.2 mg N/g dry soil, which was only 28% of the uric acid present at the beginning of the incubation.

TABLE II

CONTENT AND ISOTOPE RATIO OF NITROGEN FROM URIC ACID-CONTAINING SOIL

The purity of uric acid in a uric acid fraction was determined by comparing a spectroscopically measured uric acid content with total organic nitrogen in the fraction, and three separate analyses gave a purity of 94.4 ± 2.1%.

*Class**	*Content (mg N/g dry soil)***	*Isotope ratio (‰)***
Total N	37.5 ± 2.4 (3)	27.1 ± 0.8 (3)
NH_3-N in dried soil	11.8 ± 0.5 (5)	45.2 ± 0.04 (3)
NH_3-N in evaporate	10.4	3.9
NH_3-N in gas phase	4.4 ± 0.1 (3)	−3.8
Uric acid-N	5.7 ± 0.4 (3)	15.8 ± 0.3 (3)

* Total N is the amount of Kjeldahl nitrogen found in the soil after drying, and includes neither NH_3-N in the evaporate nor that in the gas phase. NH_3-N in the evaporate is that of the ammonia that evolved during the vacuum drying of wet soil after the end of the incubation. NH_3-N in the gas phase is that of the ammonia that left the soil during the incubation. The overall NH_3-N amounted to 26.6 mg N/g dry soil, and the weighted average of its nitrogen isotope ratio was 20.9‰.

** Values ± standard deviations, with number of determinations in parentheses.

Therefore, 72% of the acid, or 14.4 mg N/g dry soil, must have decomposed during the 10-day incubation period.

The decrease in uric acid is accompanied by an increase in ammonia. The total ammonia-nitrogen was 26.6 mg N/g dry soil, which almost equalled the amount of total soil nitrogen before the addition of uric acid. This therefore demonstrates that the degradation of the acid must soon lead to the production of ammonia. This can also be seen from the result of the experiment with the core to which no exogenous uric acid was added (UA^-); the core gave 7.7 mg N/g dry soil for the NH_3-N in dried soil and 38.0‰ for its nitrogen isotopic ratio. The ratio was 7.2‰ less than that from the uric acid-added core (UA^+), and this difference can be understood if the decomposition of uric acid resulted in the production of ammonia, as the difference between the ratios of uric acid in the UA^+ and UA^- experiments was 6.7‰.

From field observations[15], it has been noted that the soil ammonium content in a Kabushima rookery soil and in a soil from Tsubakishima, another Black-tailed Gull rookery, was highest at the surface (0–5 and 0–1 cm), where a high concentration of uric acid was found, and that its concentration decreased monotonously with the depth, the acid being absent at depths greater than 5 cm.

The nitrogen isotope ratio of uric acid in the soil both before and after the incubation was 15.8‰, which indicates that there is no substrate preference by uric acid bacteria in terms of nitrogen isotopes. The overall nitrogen isotope ratio of soil ammonia after the incubation was 20.9‰, which was 5.1‰ higher than that of the uric acid before the incubation.

The nitrogen isotope ratio of NH_3 in the gas phase was -3.8‰ and was far lower than that in wet soil (25.9‰; that is, the avarage of NH_3-N in dried soil and in evaporite). A study[27] of barnyard and feedlot soils in Texas, U.S.A., indicated that the isotope effect during the ammonia evaporation increases the nitrogen isotope ratio of the remaining inorganic nitrogen in soil by as much as 38‰. The present observations agree with that study and may explain that the generally high nitrogen isotope ratio in rookery soil[4] is not due to the decomposition of uric acid to ammonia, but rather to the isotope effect during the evaporation from soil of the ammonia produced.

It is interesting that an extraordinarily high nitrogen isotope ratio (45.2‰) was obtained from the ammonia fraction of the soil after vacuum-drying. As the present incubation was carried out under conditions of 100% humidity, which is not always the case in a natural environment, and there must be a dry day in a field, soil ammonia with such a high nitrogen isotope ratio or even higher from natural samples could be found. To simulate in nature what happens during the vacuum-drying procedure, the existence of a less humid period of time or the presence of a temporarily high pH in the liquid phase of a soil would be sufficient.

The HPLC isolation of uric acid from soil for isotopic determination would clarify the nitrogen dynamics in a bird rookery, which must be a key in establishing a unique ecosystem, often with a luxuriant plant community in contrast with the surroundings. An application of the methods described in this paper and in the accompanying paper[15] should help to elucidate the make-up of the ecosystem, and even to study the fate of animal excreta applied to a cultivated field as a fertilizer and the dynamics of the formation of fairy rings[28]. The method may also be used to study the dietary analysis of wild birds without their capture or even their sighting,

as uric acid isolated from soil retains the same nitrogen isotope ratio as that at the time of its deposition. This may further make it possible to make physiological studies of avian species in wildernesses, as the isotope ratio must reflect their physiological conditions and also their diet.

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