63.11; H, 4.98.

Demethylation of the acid with 50% HI gave the phenol which was recrystallized from EtOH to prisms, m.p. 238°(decomp.). *Anal.* Calcd. for $C_{14}H_{14}O_7$: C, 57.14; H, 4.80. Found: C, 56.67; H, 4.70.

1,4-Dihydroxy-8-oxotetrahydronaphthyl-7-acetic Acid (VIII)—3 g. of (VI) was refluxed with 10 g. of 50% HI for 15 mins. in CO_2 atmosphere, and then 50 cc. of water was added. Separated crystals (1.8 g.) were recrystallized from water to pale yellow prisms, m.p. 179°. *Anal.* Calcd. for $C_{12}H_{12}O_5$: C, 61.01; H, 5.12. Found: C, 60.64; H, 5.03.

1,4,8-Trihydroxytetrahydronaphthyl-7-acetic Acid Lactone (IV)—To a solution of 1.5 g. of (VIII) in 50 cc. of 2% aq. solution of $NaHCO_3$, 20 g. of 3% sodium amalgam was added, and the mixture was stirred in CO_2 atmosphere for 12 hrs. The aq. layer was acidified with dil. HCl and separated crystals were recrystallized from EtOH to prisms, m.p. 220~221°. The compound gave yellow coloration with $FeCl_3$. *Anal.* Calcd. for $C_{12}H_{12}O_4$: C, 65.44; H, 5.49. Found: C, 65.43; H, 5.23.

The acetate (V) was prepared with Ac_2O and H_2SO_4. Recrystallization from EtOH gave needles, m.p. 187°. *Anal.* Calcd. for $C_{16}H_{16}O_6$: C, 63.15; H, 5.30. Found: C, 63.46; H, 5.24.

Summary

2-Oxo-8,10-dihydroxy-$\Delta^{1,9;3,4}$-hexahydronaphthyl-7-acetic acid lactone underwent rearrangement to 2,4,8- and 1,4,8-trihydroxytetrahydronaphthyl-7-acetic acid lactone by dilute sulfuric acid, and to 2,4-diacetoxy-8-hydroxytetrahydronaphthyl-7-acetic acid lactone by acetic anhydride and sulfuric acid. It was concluded that when hydroquinone rearrangement was hindered a resorcinol rearrangement might take place instead of catechol rearrangement. A synthesis of 1,4,8-trihydroxytetrahydronaphthyl-7-acetic acid lactone was also described.

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64. Izumi Imaseki: Phytochemical Investigation on Cultivation of Medicinal Plants. IX.[1] On the Alkaloid Biogenesis in Datura. (2).

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In the previous communications[1,2] Shibata and Imaseki have shown by the grafting experiments that the alkaloids in Datura are formed principally in the root, though some possibility of alkaloid formation in the leaves cannot entirely be ruled out.[3,4] It was shown, moreover, that the ratio of the contents of hyoscyamine and scopolamine (hyoscine) in the root and aerial part of the plant varies during the growth.[1] Almost simultaneously, a similar observation was given by Evans and Partridge.[5]

In the present work ^{15}N-labelled ammonium sulfate was fed to *Datura tatula* L. to trace the rate of alkaloidal formation during a definite growing period, referring to the proportional contents of scopolamine and hyoscyamine in the leaves.

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1) Part VIII: S. Shibata, I. Imaseki: J. Pharm. Soc. Japan, **74**, 862(1954).
2) S. Shibata, I. Imaseki: *Ibid.*, **73**, 797(1953).
3) cf. Dawson: "Recent Advences in Enzymology," 8, 203(1948); W. O. James: "The Alkaloids," Ed. Holmes and Manske, Academic Press, Vol. **1**, p. 15(1950).
4) References to the recent works on the problems of the site of Datura alkaloid formation were cited in the footnote to the paper published by B. T. Jackson and J. M. Rowson (J. Pharm. Pharmacol., **5**, 778(1953)); W. C. Evans, M. W. Partridge: *Ibid.*, **6**, 702(1954).
5) W. C. Evans, M. W. Partridge: *Ibid.*, **5**, 772(1953).

Experimental

Material and Method—On June 10, 1954, on the 50th day of sowing, *Datura tatula* plants about 5 cm. in height were transplanted into the Wagner pots, each of which was filled with 11 kg. of infertile soil obtained from Tanashi Experimental Farm, University of Tokyo.

These were devided into 3 groups, each group consisting of 5 plants. The nutrients supplied to each pot for cultivation consisted of N : 1.0 g. ($(NH_4)_2SO_4$), P_2O_5 : 3.0 g. ($CaH_4(PO_4)_2CaSO_4.2H_2O$), and K_2O : 1.5 g. (K_2SO_4). After 40 days (July 20), in the stage of flower-bud formation, when a nitrogen deficiency symptom evidently appeared in the leaves of plants, 2.0 g./pot of ^{15}N were applied to the 2 groups of plant in the form of ammonium sulfate (4.18 atom % ^{15}N-excess) while the third group was harvested as a control.

One of the groups of the treated plants was harvested at the end of the first week (July 27) and the second group was harvested a week later (August 3). The leaf materials, after being collected, were rapidly dried, weighed, and pulverized to prepare samples for determinations of the total-N, protein-N, nonprotein-N, and the contents of hyoscyamine and scopolamine, as well as the concentration of ^{15}N in each fraction.

Procedure——The mixture of alkaloids extracted from the samples by the usual method was fractionated by the partition column chromatography[6] and each fraction obtained was titrated to give the amount of the alkaloid. Each alkaloid fraction was then made alkaline with NH_4OH and extracted with $CHCl_3$, and the extract was dried and evaporated when hyoscyamine was obtained in a crystalline form whereas scopolamine gave a syrup. The N-content of the alkaloid fraction was estimated by the micro-Kjeldahl method using a mixture of conc. H_2SO_4 (3 cc.), K_2SO_4 (2 g.), and Se (0.01 g.) for the sample containing 3~5 mg. of alkaloidal-N. The final solution which was used for the N-estimation was slightly acidified and concentrated on a boiling water bath to 1~2 cc. N_2 gas was liberated from the concentrated solution by the action of NaOBr and collected into a gas reservoir to determine ^{15}N-concentration by the Consolidated Mass Spectrometer Model 21-103 A.

Results

At the beginning of this experiment when ^{15}N-labelled ammonium sulfate was fed to the test plants, the symptom of nitrogen deficiency was observed in the leaves. However, 1 week after nitrogen fertilization general appearance of the plants showed recovery of the normal condition of growth.

Table I shows the growth and yield of each part of the plant harvested on the 7th and 14th day after ^{15}N-feeding.

Table I.

No. of week after treatment	Date	Height (cm.)	Diam. of stem (cm.)	No. of leaves	Surface area of leaf (cm^2.)	Yield (dry wt.) Leaf	Stem	Root	(g.)
0	July 20	38.2±4.7	1.05±0.08	17.7± 2.1	16.6±1.5×11.8±1.4	3.4	2.6	3.0	9.0
1	July 27	55.0±4.1	1.15±0.05	31.1± 3.2	17.6±1.7×12.7±0.9	6.2	8.8	5.4	20.4
2	Aug. 3	65.6±2.2	1.26±0.05	76.0±14.6	18.2±1.5×13.1±1.3	13.6	10.2	7.2	31.0

Table II gives the results of nitrogen analyses following ^{15}N-feeding to *Datura tatula*.

Table II.

No. of week after treatment		Total amount %	Total amount mg.	Total amount ratio	N absorbed during exptl. period ^{15}N atom % excess	N absorbed during exptl. period mg.	N absorbed during exptl. period ratio	Absorbing ratio*	Increasing ratio**
0	Total-N	4.90±0.03	166.6	100	—	—	—	0	0
	Protein-N	3.56±0.02	121.0	72	—	—	—	0	0
	Nonprotein-N	—	45.6	28	—	—	—	0	0
1	Total-N	6.21±0.03	385.0	100	1.46±0.01	134.5	100	35	56
	Protein-N	5.16±0.05	319.9	83	1.47±0.01	112.5	84	35	62
	Nonprotein-N	—	65.1	17	—	22.0	16	34	30
2	Total-N	7.54±0.03	1025.4	100	2.78±0.01	683.3	100	69	84
	Protein-N	5.74±0.04	780.6	76	2.92±0.01	545.9	80	70	85
	Nonprotein-N	—	244.8	24	—	137.4	20	57	81

* The ratio of the absorbed nitrogen to the total nitrogen in the leaves during 1 and 2 weeks of ^{15}N-feeding.

** The ratio of the nitrogen increased to the total amount of nitrogen in the leaves during 1 and 2 weeks of ^{15}N-feeding.

6) W. C. Evans, M. W. Partridge : Quart. J. Pharm. Pharmacol., **21**, 126(1948).

Table III shows the results of the determination of alkaloidal content after ^{15}N-feeding.

TABLE III.

No. of week after treatment	Alkaloid	Total amt. of each alkaloid fract. %	mg.	ratio	$\frac{\text{Alk. N}}{\text{Total-N}}\times 100$	Amount of alkaloid formed after ^{15}N-feeding: ^{15}N atom % excess	mg.	ratio	$\frac{A}{B}\times 100$ **	Formation ratio***	Increasing ratio****
0	Total	0.229	7.8	100	0.23	—	—	—	—	—	—
	Hyoscyamine	0.080	2.7	35	0.08	—	—	—	—	—	—
	Scopolamine	0.143	4.9	62	0.14	—	—	—	—	—	—
	Other bases	0.007	0.2	3	—	—	—	—	—	—	—
1	Total	0.384	23.8	100	0.30	—	1.6*	100	0.06	7	67
	Hyoscyamine	0.181	11.2	47	0.14	0.46±0.01	1.2	77	0.04	11	76
	Scopolamine	0.196	12.1	51	0.15	0.12±0.01	0.4	23	0.01	3	60
	Other bases	0.004	0.2	1	—	—	—	—	—	—	—
2	Total	0.276	37.6	100	0.18	—	10.9*	100	0.08	29	79
	Hyoscyamine	0.156	21.2	56	0.10	1.51±0.01	7.6	70	0.05	36	87
	Scopolamine	0.115	15.7	42	0.07	0.86±0.01	3.2	30	0.02	20	69
	Other bases	0.002	0.3	1	—	—	—	—	—	—	—

* The amount of scopolamine and hyoscyamine.
** A : Alkaloidal-N formed, B : Total-N absorbed after ^{15}N-feeding.
*** The ratio of the alkaloid formed by ^{15}N-feeding to the total amount of alkaloid in the leaves.
**** The ratio of the alkaloid increased after ^{15}N-feeding to the total amount of the alkaloid in the leaves.

The increasing amount of ^{15}N in each nitrogen fraction is shown in atom% excess (Fig. 1) and in mg. per plant (Fig. 2). Fig. 3 gives the change in the amount of hyoscyamine and scopolamine traced after ^{15}N-feeding.

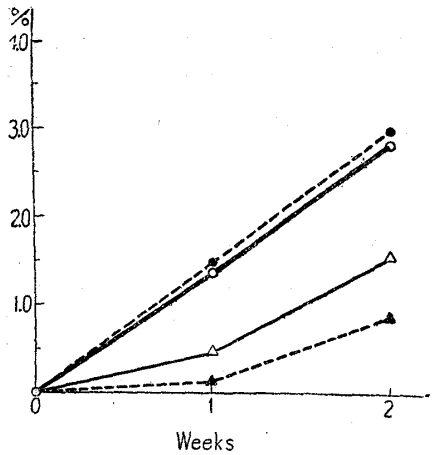

Fig. 1. ^{15}N atom% exess
—○— Total-^{15}N
--●-- Protein-^{15}N
—△— Hyoscyamine-^{15}N
--▲-- Scopolamine-^{15}N

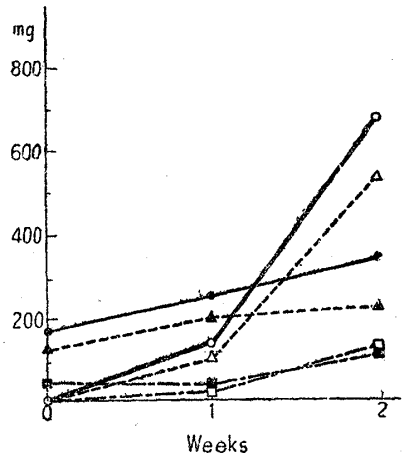

Fig. 2. Nitrogen Content in leaves (mg. per plant)
—○— ^{15}N-labelled total-N
—●— Non-labelled total-N
--△-- ^{15}N-labelled protein-N
--▲-- Non-labelled protein-N
---□--- ^{15}N-labelled non-protein-N
---■--- Non-labelled non-protein-N

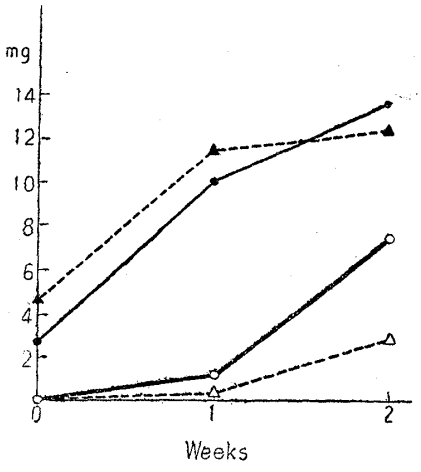

Fig. 3. Alkaloid Content in Leaves (mg. per plant)
—○— ^{15}N-labelled hyoscyamine
—●— Non-labelled hyoscyamine
--△-- ^{15}N-labelled scopolamine
--▲-- Non-labelled scopolamine

Discussion

It is evidently shown in Table III that $(^{15}NH_4)_2SO_4$ was utilized to build up the alkaloids in Datura, which showed the amount of 7% of the total alkaloids in the leaves 1 week after ^{15}N-feeding and 30% after 2 weeks.

On the other hand, the proportion of ^{15}N in the total-N and the protein-N increased steadily during the two weeks, otherwise the rates of alkaloid formation and protein biosynthesis in *Datura* were not parallel (Fig. 1).

As shown in Fig. 2, on the 7th day of ^{15}N-feeding, the amount of the protein-N in the leaves derived from ^{15}N-labelled ammonium sulfate gave an almost equal proportion with that derived from ordinary N-source which was supplied or accumulated before ^{15}N-feeding and translocated from other parts of the plant, and after two weeks the proportion of the ^{15}N-labelled protein-N reached far higher degree than that derived from ordinary N-source.

On the contrary, the amount of the ^{15}N-labelled alkaloids accumulated in the leaves was unexpectedly low in comparison to that derived from ordinary N-source, even at the end of the 2nd week of ^{15}N-feeding (Fig. 3).

Moreover, a marked difference was also observed in the quantity of hyoscyamine and scopolamine in the leaves formed from the ^{15}N-source during the two weeks' experimental cultivation: Eleven per cent of the total hyoscyamine was labelled with ^{15}N at the end of the 1st week and 36% was marked at the end of the 2nd week of ^{15}N-feeding, whereas the corresponding figures given for scopolamine were 3% and 20%, respectively.

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Summary

The rate of alkaloid formation in *Datura tatula* L. was observed employing ^{15}N as a tracer element. The up-take of ^{15}N in the alkaloid in the leaves of the test plant was preceded by that in protein. A marked difference was also shown between the rate of formation of hyoscyamine and that of scopolamine during the two weeks' experimental cultivation.

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65. **Torizo Takahashi und Fumio Yoneda**: Über die Synthese der heterozyklischen Verbindungen mit Stickstoff. XCVIII.[1] Einwirkungen von α-Halogenketonen auf 3-Nitro-4-oxypyridin sowie 3-Amino-4-oxypyridin.

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Über die Reaktionen von α-Halogenketonen mit 3-Nitro-4-oxypyridin und 3-Amino-4-oxypyridin gibt es bisher keine Angaben in der Literatur. Nachfolgend berichten wir die Ergebnisse, die wir in Versuchen hierüber errungen haben.

Das Ausgangsmaterial, 3-Nitro-4-oxypyridin (I) wurde nach dem Koenigs'schen Verfahren[2]—Nitrierung von 4-Oxypyridinnitrat mit rauch. Salpetersäure und rauch. Schwefelsäure—hergestellt.

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1) XCVII Mitt.: J. Pharm. Soc. Japan, **75**, 277 (1955).

2) E. Koenigs, K. Freter: Ber., **57**, 1189 (1924).