The Structure of Aureothin. X[1]. *Aza Derivatives of Aureothin, Isoaureothin and Desmethylisoaureothin*

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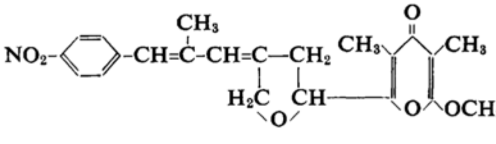
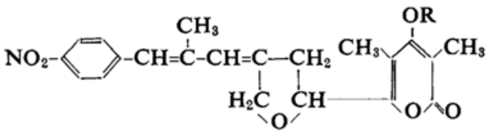
A recent publication[2] from our laboratory has indicated that the structure of aureothin was shown to be I, on a firm basis of a quantity of chemical and spectroscopic evidence. Aureothin undergoes demethylation with various acids, followed by isomerization, to give desmethylisoaureothin (II, R=H), the methylation of which, in turn leads to isoaureothin (II, $R=CH_3$) as a major product[1].

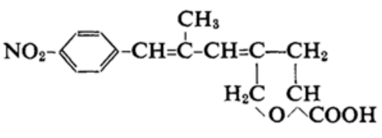
During the course of the structural investigation, it was found that I, II ($R=CH_3$) and II (R=H) reacted with ammonia and were transformed to aza-aureothin (IV), aza-isoaureothin (V) and aza-desmethylisoaureothin (VI), respectively. In the present paper, the properties of these aza-derivatives are described, being compared with those of other model compounds. Each of these aza-derivatives contains one more nitrogen, besides an original one (nitro group), and is sparingly soluble both in organic solvents and water.



(I)



(II)



(III)

Aza-aureothin (IV).—The elemental analysis of IV was incompatible with the molecular formula, expected for γ-pyrone—γ-pyridone

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[1] For Part IX of this series, see, H. Nakata, This Bulletin, in press.

[2] K. Yamada, H. Nakata and Y. Hirata, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **81**, 340 (1960).

[3] Y. Hirata, K. Okuhara, H. Nakata, T. Naito and K. Iwadare, ibid., **78**, 1700 (1957).

transformation; there remained no methoxyl group in IV. Thus, somewhat complicated reactions must take place during the formation of IV from I. It is apparent from the ultraviolet spectrum that γ-pyrone moiety is involved in the reaction, and the conjugated *p*-nitrophenyl system remains intact. Subtraction of the ultraviolet spectrum of aureothinic acid (III) from that of aza-aureothin (IV) gave an absorption spectrum due to the chromophore in question—presumably, a nitrogen-containing ring; the subtraction curves, taken in neutral and acid solutions, were different, as shown in Fig. 1.

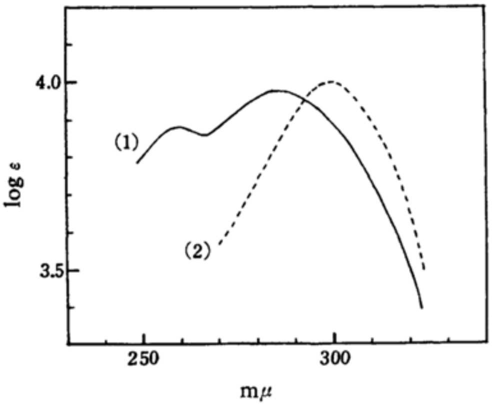


Fig. 1. Substraction curves obtained by aza-aureothin (IV)—aureothinic acid (III) in ethanol.
(1): Neutral and alkaline (0.1 N EtONa) solutions.
(2): Acidic (0.1 N HCl) solution.

The infrared spectrum showed bands at 1666(s), 1643(m), 1583(s), 1529(s), 1509(s) cm^{-1} and at 3385, 3285 and 3175 cm^{-1}, the latter group of bands suggesting the presence of N–H and/or O–H. Treatment of IV with acetic anhydride and pyridine yielded an amorphous substance, the infrared spectrum of which showed bands at 1731(s), 1666(s), 1623(s), 1596(s) and 1516(s) cm^{-1} (in chloroform). This must be an ester. The insolubility of IV in various solvents prevented us from attempting further studies on this substance, and the details of the structure are still uncertain.

Aza-isoaureothin (V).—Analytical data of V agreed with a molecular formula $C_{21}H_{22}O_5N_2$, identical with that of aza-desmethylisoaureothin (VI), but the chemical behavior, as described below, is quite different from those expected for aza-desmethylisoaureothin (VI). In this case, again, some other reaction might occur concomitantly with the pyrone—pyridone transformation. This compound is not an acidic substance but a neutral one, and an attempted acetylation only resulted in the complete recovery of the starting material. The same procedure used for IV was applied for the measurement of the ultraviolet spectrum; the results obtained by the subtraction method of the ultraviolet curves are shown in Fig. 2. In this case also, the absorption maximum in an acidic medium is situated at somewhat longer wavelength than that in neutral solution.

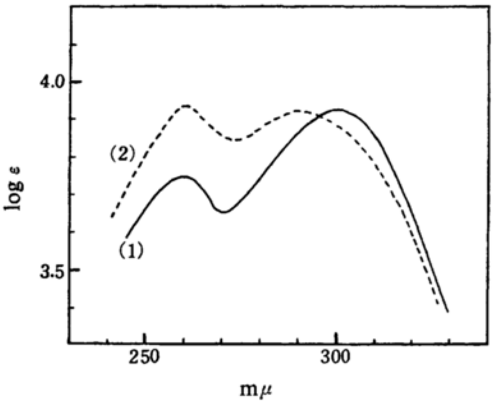


Fig. 2. Subtraction curves obtained by aza-isoaureothin (V) — aureothinic acid (III) in ethanol.
(1): Neutral and alkaline (0.1 N EtONa) solutions.
(2): Acidic (0.1 N HCl) solution.

Remarkable differences were observed in the infrared spectra between isoaureothin (II, R=CH_3) and aza-isoaureothin (V), as illustrated in Fig. 3. The following points are worth noticing; the shift of carbonyl band (1716 cm^{-1} for isoaureothin, 1660 cm^{-1} for aza-isoaureothin), and the appearance of a new band at 1554 cm^{-1} and of a series of bands at 3225, 3322, 3434 cm^{-1}.

From these spectroscopic data, it is seen that an α-pyridone ring is likely to be present in V, but the whole structural aspect of V could not yet be disclosed, owing to the same reason, as that encountered in the case of aza-aureothin (IV).

Aza-desmethylisoaureothin (VI). — Aza-desmethylisoaureothin $C_{21}H_{22}O_5N_2$, was prepared via two routes; one is based on a reaction of desmethylisoaureothin (II, R=H) with ammonia, and the other is due to the treatment of aureothin with ammonium acetate in acetic acid. Chemical and spectroscopic behavior of VI, described below, substantiates a simple pyrone-pyridone transformation. VI dissolved in aqueous alkali, although no reaction took place between VI and ethereal diazomethane (under heterogeneous condition). Acetylation of VI with acetic anhydride-pyridine gave aza-desmethylisoaureothin acetate, $C_{23}H_{24}O_6N_2$ (VII), which showed an enol acetate band at 1767 cm^{-1} (KBr).

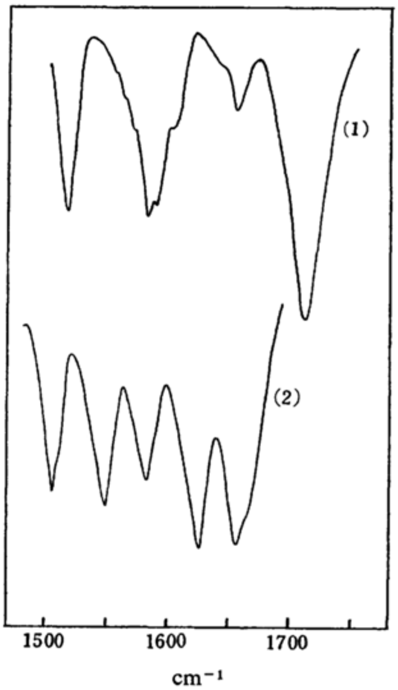


Fig. 3. Infrared spectra (KBr)
(1): Isoaureothin. (II, $R=CH_3$).
(2): Aza-isoaureothin. (V).

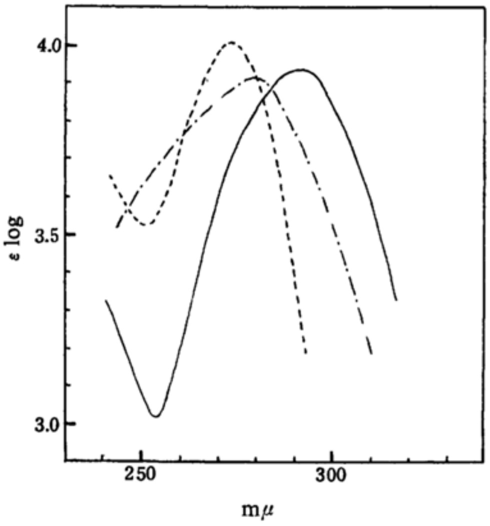
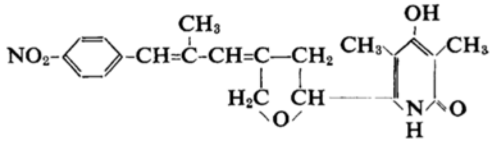


Fig. 4. Subtraction curves obtained by aza-desmethylisoaureothin (VI)—aureothinic acid (III) in ethanol.
(1): Neutral solution.
(2): Acidic (0.1 N HCl) solution.
(3): Alkaline (0.1 N EtONa) solution.

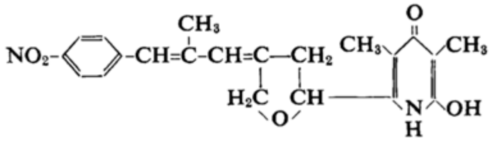
In the infrared spectrum of VI, there appeared bands at 1643(s), 1605(v. s), 1585(v. s), 1564(m) and 1510(s) cm^{-1} in 1800~1500 cm^{-1} region and a broad band ranging from 2500 cm^{-1} to 3000 cm^{-1}, which indicates the occurrence of hydrogen bonding. The position of pyridone-carbonyl band unaffected by hydrogen bonding is available from aza-desmethylisoaureothin acetate (VII); i. e., in this case, $\nu_{C=O}$ appeared at 1654 cm^{-1} (KBr).

In the ultraviolet region, the absorption due to the pyridone ring of VI had its maximum at 291 mμ in neutral solution, while acidic and alkaline conditions caused the hypsochromic displacement of the absorption (Fig. 4). The moderate solubility in chloroform made possible the ozonization of VII, which afforded aza-desmethylisoaureonone acetate, $C_{13}H_{15}O_5N$ (VIII). This product VIII, now free from the conjugated *p*-nitrophenyl system, showed an absorption maximum at 304 mμ in the ultraviolet region, and bands at 1762(s), 1658(s), 1640(sh.) and 1577(w) cm^{-1} in the infrared region.

Structure of Aza-desmethylisoaureothin.—Both types VIa and VIb would be possible for VI, since enolization can occur in two directions⁴⁾.



(VIa)



(VIb)

A considerable amount of data⁵⁾ concerning the infrared spectra of various α-pyridones is now at hand. The condition of the measurement (solid or solution) has a fairly large influence on the position of carbonyl absorption. The ranges quoted for carbonyl absorption in the solid state are rather wide (1630~1680 cm^{-1})⁵⁾, whereas in solution, it has been reported that carbonyl frequencies fell into a somewhat narrow range (1654~1687 cm^{-1})⁶⁾.

As to γ- pyridones, the data so far are not sufficient to obtain the detailed information about $\nu_{C=O}$; in general, $\nu_{C=O}$ is expected to appear in

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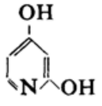
4) One more possibilty, the diketo-form, is excluded on the ground of its relation to the ultraviolet spectrum.

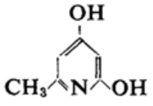
5) (a) W. F. Cockburn and L. Marion, *Can. J. Chem.*, **29**, 13 (1951). (b) F. Ramirez and A. P. Paul, *J. Org. Chem.*, **19**, 183 (1954); *J. Am. Chem. Soc.*, **77**, 1035, 3377 (1955). (c) J. A. Gibson, W. Kynaston and A. S. Lindsey, *J. Chem. Soc.*, **1955**, 4340. (d) F. Bohlman, A. Englisch, N. Ottawa, H. Sander and W. Weise, *Chem. Ber.*, **89**, 792 (1956). (e) K. Hoegerle and H. Erlenmeyer, *Helv. Chim. Acta*, **39**, 1203 (1956). (f) R. H. Wiley and S. C. Slaymaker, *J. Am. Chem. Soc.*, **78**, 2393 (1956).

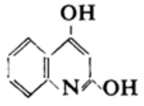
6) S. F. Mason, *J. Chem. Soc.*, **1957**, 4874.

the region 1630∼1650 cm^{-1} (solid and solution)[7]. According to Mason, the C=O band of the α-pyridones is located at higher frequency than that of γ-isomers, whilst the reverse relation is observed regarding the N–H band[6]. Further, it was noticed[7d] that in α-pyridones, the intensity of $\nu_{C=O}$ is usually stronger than that of $\nu_{C=C}$, whereas in γ-pyridones carbonyl absorption appears as a relatively weak band.

The following compounds may serve as good models of aza-desmethylisoaureothin (VI), in relation to the problem of tautomerism; 2, 4-dihydroxypyridine (IX)[6], 6-methyl-2, 4-dihydroxypyridine (X) and 2, 4-dihydroxyquinoline (XI)[6].

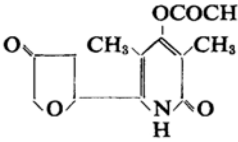

(IX)

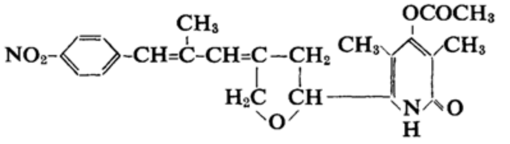

(X)


(XI)

As to dihydroxypyridine (IX) it was believed that an α-pyridone form was predominant in aqueous solution[8] on the ground of the ultraviolet evidence, while the possible coexistence of α, γ-forms was suggested for IX in the solid state[9]. As for 6-methyl-2, 4-dihydroxypyridine (X), it is insufficiently soluble in chloroform to give an infrared spectrum in solution, but in the solid state (KBr), there appeared bands at 1658(s), 1639(s) and 1607(s) cm^{-1} and a broad band near 3000 cm^{-1} indicating a hydrogen bond. With the information about the diagnosis of α, γ-tautomers at hand, it is concluded that the tautomer of α-pyridone form occurs in X. An α-pyridone structure was inferred[7d] for XI itself and its acetate, but not established firmly as yet.

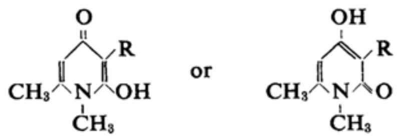
With the help of the generalization described above, the structural examination of aza-desmethylisoaureothin (VI) will be made. The structure VIII is assigned to aza-desmethylisoaureonone acetate, the ozonisation product, on the basis of the position (1658 cm^{-1}) and the strong intensity of the pyridone carbonyl band. A strong band at 1762 cm^{-1} in VIII is due to two carbonyls of enol acetate and five-membered ketone of tetrahydrofuran ring; two bands at 1640 and 1557 cm^{-1} are ascribed to the unsaturation of the pyridone ring[10]. Establishment of VIII for the ozonisation product leads to structure VII for aza-desmethylisoaureothin acetate.


(VIII)


(VII)

The disagreement in the position of the carbonyl band of aza-desmethylisoaureothin (1643 cm^{-1}) with that of the model compound (X, 1658 cm^{-1}), taken in conjunction with the weak intensity in the former $\nu_{C=O}$, might be in favor of the γ-pyridone structure VIb for this derivative of the natural product. However, in aza-desmethylisoaureothin, the intermolecular (or intramolecular) interaction would be more complicated than in a simple molecule, and the overlappings of the bands due to benzene ring with those of the pyridone ring make it difficult to compare exactly the intensities between $\nu_{C=O}$ and $\nu_{C=C}$ in the pyridone ring. Such circumstances give rise to difficulty in making the discrimination between (Va) and Vb, solely on the basis of the infrared spectrum.

The unusual hypsochromic shift of the ultraviolet absorption maximum in alkaline solution was recognized as a characteristic feature of the hydroxypyridones (A), distinguishing the type (A) from other types of hydroxypyridone[11]. As is expected, the model compound IX behaves similarly (see Fig. 4).


(A): R=H or C_2H_5

A good paralellism of the ultraviolet spectra between the pyridone part of VI and the model X was observed, in regard to the magnitudes

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[7] (a) R. Adams, V. V. Jones and J. L. Johnson, *J. Am. Chem. Soc.*, **69** 1810 (1947); (b) N. J. Leonard and D. M. Locke, ibid., **77**, 1852 (1955); (c) P. I. Ittyeran and F. G. Mann, *J. Chem. Soc.*, **1958**, 467; (d) H. Shindo, "Infrared Absorption Spectrum Series No. 3" Nankodo, Tokyo, (1957), p. 186.

[8] H. J. den Hertog and D. J. Buurman, *Rec. trav. chim.*, **75**, 257 (1956). Cf. H. M. Woodburn and M. Hellman, ibid.. **70**, 813 (1951).

[9] There was recorded in the solid state, a pair of bands, 1661 and 1635 cm^{-1} for IX (1662 and 1628 cm^{-1} for (XI)), upon which the presumption of the coexistence of α, γ-tautomers was based. Alternative interpretation of the spectrum, however, seems to be more reasonable; both IX and XI exist simply as an α-pyridone form on the assumption that bands, 1635 and 1628 cm^{-1} are respectively, associated with the unsaturated bonds of the ring.

[10] The positions of bands associated with the unsaturated bonds of the ring are very sensitive to the substituents[5f]; therefore, these bands are not so useful for the identification of the pyridone ring.

[11] J. A. Berson, W. M. Jones and S. L. F. O'callaghan, C. S. J., *J. Am. Chem. Soc.*, **78**, 622 (1956).

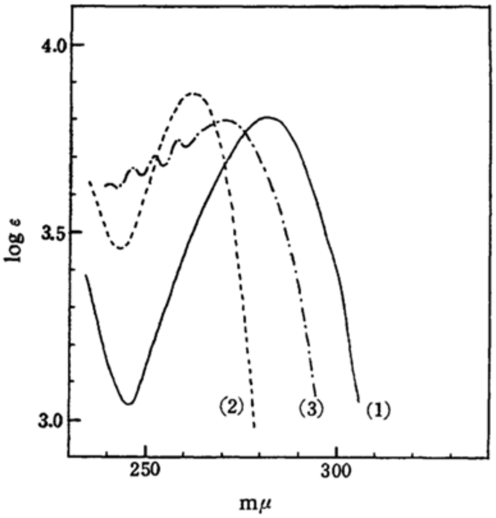

Fig. 5. Ultraviolet spectra of 6-methyl-4-hydroxy-2-pyridone (X) in ethanol.
(1): Neutral solution.
(2): Acidic (0.1 N HCl) solution.
(3): Alkaline (0.1 N EtONa) solution.

of the hypsochromic shifts and of the relative intensities. It is inferred that α-pyridone form VIa predominates in aza-desmethylisoaureothin in solution, on the analogy of the preferential α-tautomer in the cases of the models IX and X. Furthermore, the absorption at 292 mμ due to the pyridone part of VI is regarded as a reasonable position of α-pyridone ring[12].

Experimental

Aza-aureothin (IV).—One gram of aureothin was added in a mixture of ethanol (40 ml.) and aqueous ammonia (10 ml.), and the suspension was kept at 90∼95°C in a sealed tube for 10∼12 hr. Aureothin gradually dissolved with heating, and after several hours a small number of crystals began to appear. The solution on cooling to room temperature deposited yellow crystals, which were filtered and recrystallized from a large amount of ethanol, m. p. 230∼231°C. No methoxyl group was detected by Zeisel's method; the ultraviolet spectrum was measured in neutral ethanol, by assuming 380 for its molecular weight: $\lambda_{\max}$ 345 mμ (log ε 4.25), 290 mμ (log ε 4.15).

Found: C, 64.75; H, 5.68%.

A suspension of 20 mg. of aza-aureothin was heated in acetic anhydride (5 ml.) and pyridine (1 ml.) at 80∼90°C for thirty minutes. Water was added, to give the yellow precipitate, easily soluble in chloroform and ethanol.

Aza-isoaureothin (V).—A suspension of 1 g. of isoaureothin in 50 ml. of ethanol-aqueous ammonia (4 : 1) was heated at 90∼95°C in a sealed tube for 18 hr. Complete dissolution of isoaureothin was observed within the initial five hours, and then crystallization of V slowly began to take place. As the heating was continued, the solution became dark red. The product was collected and treated with 20 ml. of hot ethanol, in order to remove remaining isoaureothin. Recrystallization from a fairly large amount of dioxane gave 360 mg. of V in yellow prisms, m. p. 228∼229°C. The ultraviolet spectrum was measured in neutral ethanol with the assumption of 380 of molecular weight: $\lambda_{\max}$ 343 mμ (log ε 4.25), 253 mμ (log ε 4.25).

Found: C, 65.84, 65.62; H, 5.99, 5.97; N, 7.33%.

A mixture, which consisted of 20 mg. of V, 8 ml. of acetic anhydride and 1 ml. of pyridine, was heated at 80∼90°C for 1 hr. to give a clear solution. Upon cooling, the yellow needles separated. These were identified as starting material V.

Aza-desmethylisoaureothin (VI). — Though aza-desmethylisoaureothin was obtained by a procedure similar to that used for the preparation of IV and V, an efficient method, starting from aureothin, was developed, to give an excellent yield of (VI). A suspension of 1 g. of aureothin in 10 ml. of acetic acid containing 3 g. of ammonium acetate was gently refluxed for 4 hr. To the cooled solution, 200 ml. of water was added, when a brown oily layer at once separated and solidified. The liquid layer was removed by decantation, and the residue turned to a crystalline substance by the addition of 50ml. of hot ethanol. Washed with hot ethanol three times, the product was recrystallized from dimethylformamide and weighed 250 mg., m. p. 238∼239°C; ultraviolet spectrum: $\lambda_{\max}$ 341 mμ (log ε 4.25), 296 mμ (log ε 4.16), 245 mμ (log ε 4.18) (ethanol as solvent).

Found: C, 65.76, 65.48; H, 5.95, 6.19; N, 7.63. Calcd. for $C_{21}H_{22}O_5N_2$: C, 65.95; H, 5.80; N, 7.33%.

To a solution of 1 ml. of pyridine and 5 ml. of acetic anhydride, 20 mg. of VI was suspended. The mixture became a clear solution when kept at 70∼80°C for 30 min. Yellow precipitates were obtained from the cooled solution, by the addition of 20 ml. of water. They were recrystallized from ethanol whereupon yellow needles of VII with m. p. 211∼213°C were obtained (15 mg.): ultraviolet spectrum: $\lambda_{\max}$ 325 mμ (log ε 4.32), 238 mμ (log ε 4.30); infrared spectrum: 1767(v. s), 1652(s), 1610(m), 1591(s) and 1516(s)cm^{-1} (KBr).

Aza-desmethylisoaureonone Acetate (VIII).—A solution of 350 mg. (0.83 m. mol.) of aza-desmethylisoaureothin acetate (VII) in 15 ml. of chloroform was cooled to −5∼−10°C and ozone was passed through this solution, until about one equivalent amount (0.95 m. mol.) of ozone was absorbed. On removal of solvent under reduced pressure, a gummy residue was obtained, which was treated with 10 ml. of water in a flask provided by a reflux condenser at 60°C. The aqueous layer in the flask was separated from the solid residue by decantation and the aqueous fraction was distilled under reduced pressure to afford a slightly yellow residue, which on recrystallization from methanol, weighed 10 mg.

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[12] The ultraviolet technique is not necessarily reliable for the discrimination of α-pyridones from their γ-isomers, in spite of the fact that α-pyridone itself absorbs at a much higher wave length (297 mμ) than γ-isomer (256 mμ); the substitution of the ring often causes an outstanding change in the spectrum.

with m. p. 208~209°C. No volatile carbonyl compound could be detected by distilling the aqueous fraction into a 2, 4-dinitrophenylhydrazine solution. The solid residue, obtained from the decomposition of ozonide, consisted of two components; one was easily soluble in methanol, whereas the other was sparingly soluble. The former was identified as *p*-nitro-α-methylcinnamaldehyde by its melting point and infrared spectrum. The latter, sparingly soluble in methanol, was recrystallized from methanol and gave 70 mg. of colorless needles, which turned out to be identical with the crystals obtained from the water soluble fraction, as described before. The total amount was 80 mg., m. p. 209~210°C; ultraviolet spectrum: $\lambda_{\max}$ 304 mμ (log ε 3.85), 233 mμ (log ε 3.81).

Found: C, 58.76, 58.86; H, 5.84, 5.98; N, 5.66, 5.45. Calcd. for $C_{13}H_{15}O_5N$: C, 58.83; H, 5.70; N, 5.28%.

Summary

Aureothin (I), isoaureothin (II, R=CH_3) and desmethylisoaureothin (II, R=H) reacted with ammonia in a somewhat drastic way, and gave aza-derivatives respectively, some properties of which are described. Of these, aza-desmethylisoaureothin was shown to be VI containing tautomeric α, γ-dihydroxypridine, on a basis of chemical and spectroscopic evidence.

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