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THE STRUCTURE OF AUREOTHIN, A NITRO COMPOUND OBTAINED FROM *STREPTOMYCES THIOLUTEUS*

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Abstract—The structure of aureothin, the third nitro compound obtained from nature, has been elucidated as I. Several reactions of aureothin and its derivatives are described.

AUREOTHIN, a by-product of the antibiotic aureothricin, is a yellow toxin isolated from the culture of *Streptomyces thioluteus*.[1] The structural studies have been carried out in our laboratory in the last few years and the results were reported in several communications. A summary of past studies together with some additional observations are reported herewith.[2]

Chemical nature and functional groups[3,4]

Aureothin (I), $C_{22}H_{23}O_6N$, m.p. 158°, forms yellow prisms soluble in methanol, ethanol, acetone, chloroform, and tetrahydrofuran, and insoluble in water and non-polar solvents; $[\alpha]_D^{18}$ 51° (in chloroform); λ_{max}^{EtOH} 257 mμ (log ε 4·39), 346 mμ (log ε 4·27); one methoxyl group (Zeisel); two =C—C(CH_3)= groups (modified method of Karrer[5]); no acetyl group. The ferric chloride reaction is negative, it is not acetylated, and an infra-red absorption around 3300 cm^{-1} is absent; thus it contains no hydroxyl group . On the other hand, a positive nitro group reaction and infra-red absorptions at 1505 and 1332 cm^{-1} suggest the presence of a nitro group.

Isomerization

When aureothin (I) is warmed in ethanolic mineral acids, the methoxyl group is easily demethylated to give desmethylisoaureothin (II), $C_{21}H_{21}O_6N$, yellow needles, m.p. 198°, p*K*a 4·6 (in 50% aqueous ethanol).[3,4] Methylation of II with diazomethane yielded an isomer of aureothin, isoaureothin (III), $C_{22}H_{23}O_6N$, m.p. 148°.[4] Acetylation of II with acetic anhydride and pyridine afforded the non-acidic acetate (IV), m.p. 182–184°, possessing an infra-red band of enol acetate at 1772 cm^{-1} (chloroform) or 1758 cm^{-1} (Nujol).[4] This was readily reconverted into the original compound (II) upon hydrolysis. Gradual addition of a dilute solution of diazomethane to II gave a mixture of aureothin (I) and isoaureothin (III),[6,7] and the action of dimethyl sulfate on the sodium salt of II gave aureothin (I) with a fairly good yield.[8] Hydrochloric acid

[1] K. Maeda, *J. Antibiotics* **A6**, 137 (1953).
[2] The chemistry of aureothin has recently been reviewed in Japanese. Y. Hirata and H. Nakata, *Jikken Kagaku Koza* Vol. 22, Chap. 16, Maruzen Company Ltd., Tokyo (1958).
[3] Y. Hirata, K. Okuhara and T. Naito, *Nature, Lond.* **173**, 1101 (1954).
[4] Y. Hirata, K. Okuhara, H. Nakata, T. Naito and K. Iwadare, *J. Chem. Soc. Japan* **78**, 1700 (1957).
[5] Y. Hirata, *J. Chem. Soc. Japan* **68**, 104 (1497).
[6] H. Nakata, S. Takahashi, K. Yamada and Y. Hirata, *Tetrahedron Letters* No. 16, 9 (1959).
[7] H. Nakata, *Bull. Chem. Soc. Japan* **33**, 1688 (1960).
[8] H. Nakata, *Bull. Chem. Soc. Japan* **33**, 1693 (1960).

treatment of isoaureothin (III) did not reconvert it into the desmethylisoaureothin (II).[4]

The carbonyl absorptions of I and II are at 1668 and 1682 cm^{-1} (chloroform), respectively, whereas those of the methylated III and the acetylated IV are at 1700 and 1710 cm^{-1}, respectively. These shifts show the presence of a hydrogen bonding in II. The marked difference between the carbonyl absorptions of I and III suggest that the carbonyl group is involved in the isomerization.

Oxidation

Oxidation of aureothin (I) with alkaline potassium permanganate and with chromic acid gave *p*-nitrobenzoic acid and *p*-nitro-α-methylcinnamaldehyde, respectively.[3,4] Ozonolysis of I produced formaldehyde and a small amount of methyl pyruvate in addition to *p*-nitrobenzaldehyde and *p*-nitro-α-methylcinnamaldehyde.[4] Identical results were obtained with isoaureothin (III) excepting that the yield of methyl pyruvate was considerably higher. Ozonolysis of tetrahydroaureothin derivatives does not give formaldehyde.

Reduction[4]

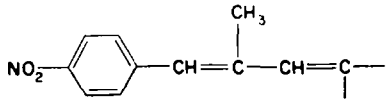
Reduction of I, II and III with ferrous salts or aluminium amalgam gave aureothamine (V), $C_{22}H_{25}O_4N$, m.p. 130–131°, desmethylisoaureothamine (VI), $C_{21}H_{23}O_4N$, m.p. 176–178°, and isoaureothamine (VII), $C_{22}H_{25}O_4N$, m.p. 134–136°, respectively, in which the nitro groups are reduced to amino groups. The Clemmensen reduction of I gave the hydrochloride of VI. Reduction of II with zinc, acetic anhydride and pyridine resulted in reduction of the nitro group and acetylation to afford N,O-diacetyl desmethylisoaureothamine, m.p. 211–212°. Isomerizations similar to these in the original nitro compound occurred readily when aureothamine (V) and N-acetyl aureothamine were treated with hydrochloric acid, and the products were desmethylisoaureothamine (VI) and its N-acetyl derivative, respectively.

When aureothin (I) and isoaureothin (III) were catalytically reduced in ethanol over platinum oxide, 5 moles of hydrogen were absorbed and the tetrahydro derivatives in which the nitro groups and two double bonds had been reduced were obtained. Tetrahydroaureothamine (VIII), $C_{22}H_{29}O_4N$, m.p. 137–138°, forms colourless plates.

Chromophores in aureothin and isoaureothin[9]

The above results suggest the partial structure (A) for aureothin and isoaureothin, and it is also apparent that the two compounds contain a carbonyl group which differs widely in its nature.

Synthetic model compounds were employed to check whether the chromophoric



(A)

group (A) was conjugated to this carbonyl group. The results are summarized in Tables 1 and 2.

If the *p*-nitrophenyl group is conjugated to the carbonyl group, the observed

[9] T. Naito, Y. Hirata, K. Okuhara and K. Iwadare, *J. Chem. Soc. Japan* **79**, 374 (1958).

TABLE 1. ULTRA-VIOLET SPECTRA OF SYNTHETIC MODEL COMPOUNDS

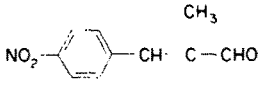
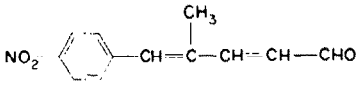
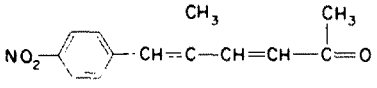
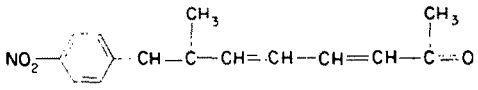
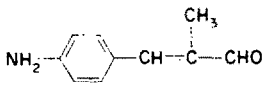
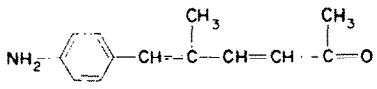
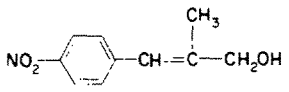
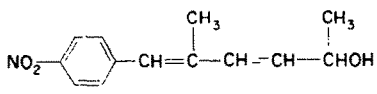
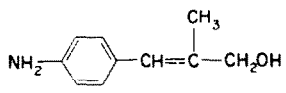
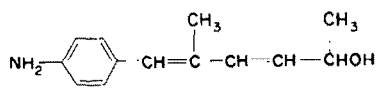
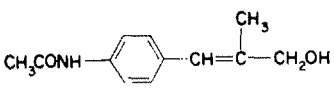
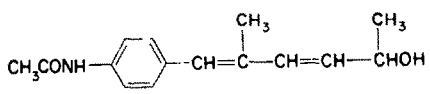
	Compound	m.p. (°C)	λ_{max}^{EtOH} (mμ)	log ε
(1)		116–117	301	4·29
(2)		141–143	323	4·33
(3)		116–118	327	4·32
(4)		135–137	355	4·35
(5)		178–180	359	4·34
(6)		131–133	262 377	4·14 4·31
(7)		46–47	221 311	4·06 4·09
(8)		86–88	243 340	4·20 4·24
(9)		82–83	271	4·27
(10)		84–86	304	4·47
(11)		123–125	268	4·25
(12)		123–125	296	4·50

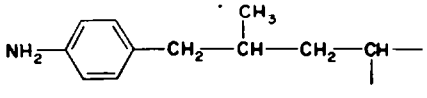
TABLE 2. ULTRA-VIOLET SPECTRA OF NATURAL COMPOUNDS

Compound	λ_{max}^{EtOH} (mμ)	log ε
(1) Nitro compounds:		
aureothin (I)	257	4·39
	346	4·27
desmethylisoaureothin (II)	248	4·26
	346	4·30
isoaureothin (III)	250	4·17
	335	4·19
O-acetyl desmethylisoaureothin (IV)	248	4·14
	335	4·23
(2) Amino derivatives:		
aureothamine (V)	241	4·24
	309	4·41
desmethylisoaureothamine (VI)	297	4·44
isoaureothamine (VII)	305	4·55
(3) N-Acetyl derivatives:		
N-acetyl aureothamine	300	4·63
N,O-diacetyl desmethylisoaureothamine	302	4·62
N-acetyl isoaureothamine	301	4·44
(4) Tetrahydro derivatives:		
tetrahydroaureothamine (VIII)	243	4·24
tetrahydroisoaureothamine	292	3·92

ultra-violet maximum would require three intervening double bonds, as in the compound (4) in Table 1. On the other hand, if it is not conjugated, then two double bonds would be required, as in (8). Models show that the maxima of the nitro compounds lie at shorter wavelength in comparison with the amino compounds when the nitrogenic chromophore is conjugated to the carbonyl group, e.g. (1) and (5), or (3) and (6). Since the natural compounds show the reverse tendency, it may be concluded that the p-nitrophenyl group is isolated from the carbonyl.

Subtraction of the ultra-violet absorption curves of these model compounds from those of the respective natural compounds gives the absorption spectra of the residual fragment containing the carbonyl group. The curves for this unknown chromophore have maxima at 250–260 mμ (log ε 4·0–4·1) and at 290–300 mμ (log ε 3·8–3·9), respectively, for aureothin and isoaureothin.

Similar subtractions of the absorption curves of *p*-toluidine and its derivatives from those of tetrahydroaureothamine (VIII) and its derivatives result in curves identical with those obtained above. This suggests that the conversion of (A) to the tetrahydroamino chromophore (B) is involved in the catalytic reduction and that the unknown chromophore remains intact (*vide infra*).

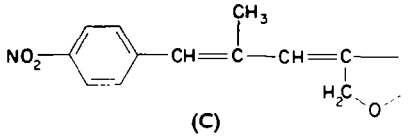


(B)

Ozonolysis of aureothin, isoaureothin, and their derivatives[10]

As was previously mentioned, the partial structure (A) for aureothin (I) and isoaureothin (III) was demonstrated by the production of *p*-nitro-α-methylcinnamaldehyde by ozonolysis. In order to identify the other structural units, ozonolyses of various derivatives were undertaken. The amino derivatives were converted to the acetates or benzoates prior to ozonolysis.

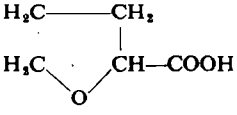
Formaldehyde is produced from aureothin (I) and isoaureothin (III) but not from the tetrahydro derivatives such as N-acetyl tetrahydroaureothamine, N-benzoyl tetrahydroaureothamine, and the corresponding iso-derivatives. The formaldehyde does not originate from a terminal methylene group since the number of C—CH_3 group remained constant before and after catalytic reduction, and since infra-red bands characteristic of terminal methylene group and gem-dimethyl group were undetectable in the curves of the original and reduced derivatives, respectively. Accordingly, it was presumed that the formaldehyde originated from the abnormal ozonolysis of an allylic ether system.[11] The position of the allylic double bond is indicated by the fact that the formaldehyde is not produced from the tetrahydro derivatives. Thus, the partial structure of aureothin may be expanded to the following:



(C)

Ozonolysis of N-benzoyl tetrahydroaureothamine, m.p. 171–172°, gave a carboxylic acid (IX) with relatively high yields; *p*-bromo-phenacyl ester, $C_{21}H_{24}O_2N$-$COOCH_2COC_6H_4Br$-*p*, m.p. 145–146°. The infra-red bands of the carboxyl group of IX were at 1770 and 1723 cm^{-1} (monomeric and dimeric, respectively, in chloroform).

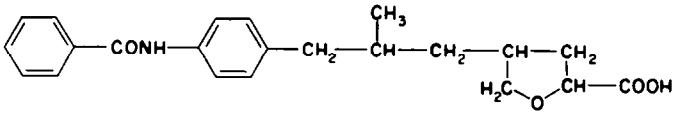
TABLE 3. INFRA-RED SPECTRA OF α-ALKOXY ACIDS

Compound	νC=O cm^{-1} ($CHCl_3$) monomer	dimer
$CH_3CH_2CH_2CH_2$—O—CH_2COOH	1780	1733
CH_3CH_2—O—CH_2COOH	1777	1733
CH_3CH_2CH(O—CH_2CH_3)—COOH	1766	1721
	1768	1723
N-Benzoyl aminotetrahydroaureothinic acid (IX)	1770	1723
Aureothinic acid (X)	1769	1721

[10] H. Nakata, Y. Hirata, K. Okuhara, K. Yamada, T. Naito and K. Iwadare, *J. Chem. Soc. Japan* **79**, 379 (1958).

[11] P. S. Bailey, *Chem. Rev.* **58**, 947 (1958).

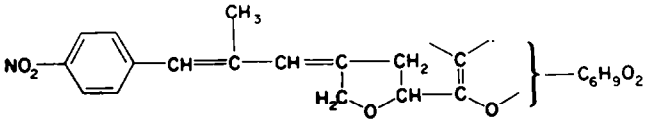
This high frequency suggested the preseence of an alpha-located electron attracting group, hence in the present case that of an ethereal function. This was confirmed by comparisons of infra-red spectra of several model compounds, as is shown in Table 3. The same behaviour of their *p*-bromophenacyl esters[12] also support the α-alkoxy acid structure. Taking into account the partial structure (C) and the fact that the acid (IX) possesses one C—CH_3 group, the above consideration leads to the following structure;



IX

The infra-red band of 1110 cm^{-1} is consistent with the presence of a tetrahydrofuran ring. This proves that, as previously mentioned, the double bonds reduced by the catalytic hydrogenation are those conjugated to the *p*-nitrophenyl group.

Furthermore since, the carboxylic acid is produced in fairly good yields only by ozonolysis, it is inferred that an enol ether linkage is present; thus, the structure of aureothin may be formulated as follows:

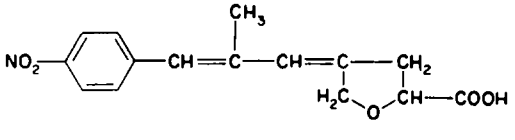


(D)

This structural unit is also common to isoaureothin since the same acid (IX) is obtained by the ozonolysis of N-benzoyl tetrahydroisoaureothamine, m.p. 165–166°.

Hydrogen peroxide oxidation of aureothin and isoaureothin[13]

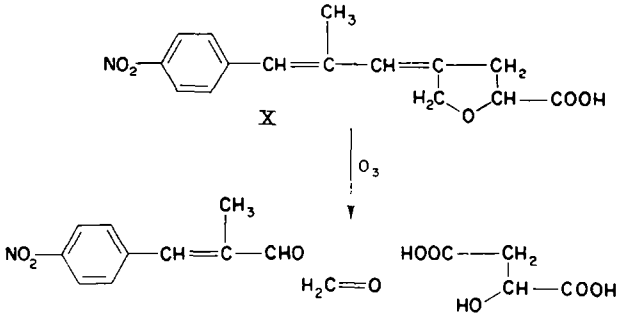
Oxidation of aureothin (I) and isoaureothin (III) with alkaline hydrogen peroxide gave aureothinic acid (X), $C_{15}H_{15}O_5N$, m.p. 147–149°. The ultra-violet absorption maximum of 249 mμ (log ε 4·14) and 345 mμ (log ε 4·20) showed that the partial structure (A) remained intact. The infra-red bands corresponding to the carboxyl group were at 1769 and 1721 cm^{-1} (monomeric and dimeric, respectively, in chloroform) or 1716 cm^{-1} (in KBr disk.) The methyl ester, m.p. 118–119°, exhibited two bands at 1758 and 1743 cm^{-1} in the ester carbonyl region in carbon tetrachloride solution. As in the case of the acid (IX), these spectroscopic properties suggested the presence of an α-ether linkage.[12] The acid (X) contains only a single C—CH_3 group and is optically active; this leads to the following structure:



X

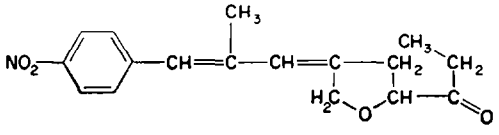
[12] H. Nakata, S. Matsuo and Y. Hirata, *Bull. Chem. Soc. Japan* **33**, 1458 (1960).
[13] Y. Hirata, H. Nakata and K. Yamada, *J. Chem. Soc. Japan* **79**, 390 (1958).

Ozonolysis of the acid gave *p*-nitro-α-methylcinnamaldehyde, formaldehyde, and malic acid (identified as its *p*-bromophenacyl ester). All of the carbon atoms may be accounted for by these three products, and accordingly, the structure (X) is rigorously confirmed. The production of formaldehyde and malic acid clearly indicated the abnormal ozonolysis of the allylic ether system in (X).



X

From the neutral fraction of the hydrogen peroxide oxidation product of aureothin, aureothinic ketone (XI) was isolated $C_{17}H_{19}O_4N$, m.p. 103–105°. Chromic acid oxidation yielded propionic acid. Thus, the structure is as follows:

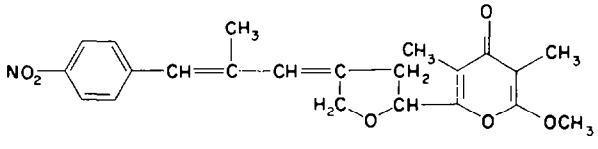


XI

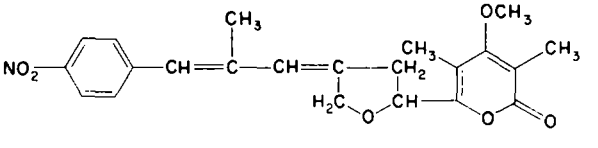
Alkaline degradation of aureothin and isoaureothin[14]

Decomposition of aureothin or isoaureothin with 1·5 N alkali under nitrogen yielded diethyl ketone as the volatile product. This showed the relative position of two methyl groups in the remaining part of the molecule.

The above results together with consideration of the partial structure (D) lead to the following structure (I) containing a γ-pyrone ring for aureothin and the alternative α-pyrone structure (III) for isoaureothin. Differentiation of the γ- and α-pyrone



I



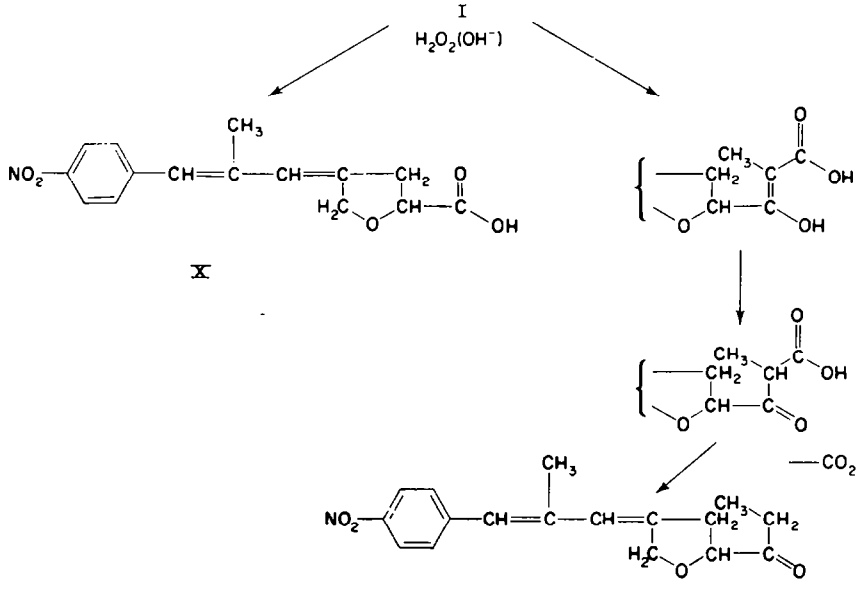
III

[14] K. Yamada, H. Nakata and Y. Hirata, *J. Chem. Soc. Japan* **81**, 340 (1960).

structure is based on comparisons of the ultra-violet and the infra-red absorptions of I and III with those of several model compounds.

A pyrone structure is suggested from indications of its aromaticity such as resistance to catalytic hydrogenation and from the subtracted ultra-violet curves (vide supra). The results of oxidation with hydrogen peroxide can also be explained satisfactorily.[13]

Taking into consideration the electron distribution in the γ-pyrone ring, the

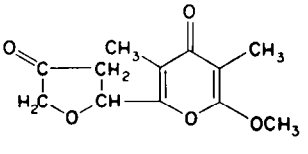


production of a small amount of methyl pyruvate by ozonolysis of aureothin (I) is also explicable.[15]

Quantitative ozonolysis of aureothin and isoaureothin[14]

Confirmation of the structure I for aureothin and III for isoaureothin was given by the quantitative ozonization reactions.

Ozonolysis of aureothin (I) with 1 mole of ozone gave the previously known *p*-nitro-α-methylcinnamaldehyde and aureonone (XII), $C_{12}H_{14}O_5$, m.p. 153–155°. The ultra-violet absorption maximum of XII was 248 mμ (log ε 3·84) and 263 mμ (log ε 3·90), which was consistent with the γ-pyrone structure but not with the α-pyrone structure. The infra-red band of 1764 cm^{-1} (in chloroform) is ascribed to the carbonyl

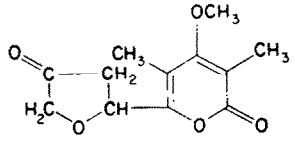


XII

[15] Ref. 11, p. 968.

group of tetrahydrofuran ring and two strong bands at 1666 and 1595 cm^{-1} are characteristic absorption of a γ-pyrone ring.

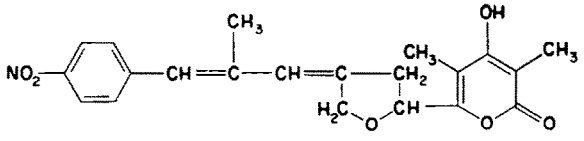
The corresponding derivative, isoaureonone (XIII), was also obtained from isoaureothin (III). The ultra-violet (λ_{max}^{EtOH} 294 mμ) and the infra-red ($\nu_{max}^{CHCl_3}$ 1764, 1704 1653 and 1567 cm^{-1}) absorptions were consistent with the α-pyrone structure.



XIII

The structure of desmethylisoaureothin

As was previously mentioned, aureothin (I) is easily demethylated with mineral acids to give desmethylisoaureothin (II). Spectroscopic properties indicated the 4-hydroxy-α-pyrone structure (II) for this demethylated product.[14]

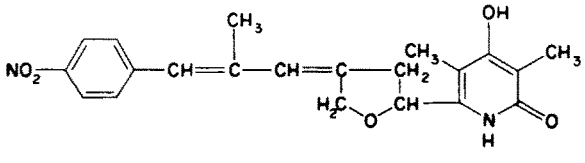


II

While the methylation of (II) with diazomethane under the usual condition gave isoaureothin (III) almost exclusively,[4] the same diazomethane methylation of II under certain specific experimental conditions gave an appreciable amount of aureothin (I).[6,7] On the other hand, the methylation of the sodium salt of II with dimethyl sulphate yielded a mixture of I and III.[8] These interconversions provided an additional and rigorous evidence of the structural relationships between aureothin (I) and isoaureothin (III).

Aza-derivatives of aureothin, isoaureothin and desmethylisoaureothin[16]

As expected for pyrone structures, the action of ammonia or of ammonium acetate in acetic acid on aureothin (I), desmethylisoaureothin (II), and isoaureothin (III) led to derivatives presumably containing pyridone rings. The reactions of I and of III were complicated and the results were inconsistent with the simple pyrone–pyridone transformation, but the product from II, aza-desmethylisoaureothin (XIV), $C_{21}H_{22}O_5N_2$, m.p. 238–239°, can be represented as follows:

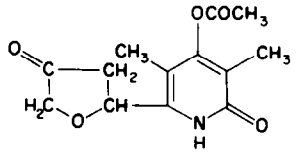


XIV

[16] K. Yamada, H. Nakata and Y. Hirata, *Bull. Chem. Soc. Japan* **33**, 1298 (1960).

The ultra-violet and the infra-red absorptions of XIV were in good agreement with synthetic pyridone derivatives.

Similar to aureonone (XII) and isoaureonone (XIII), ozonolysis of aza-desmethyl-

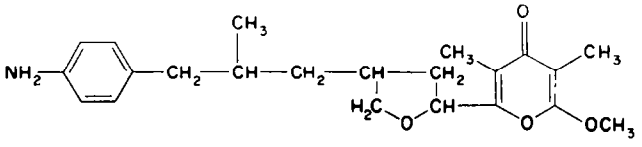


XV

isoaureothin acetate gave aza-desmethylisoaureonone acetate (XV), $C_{13}H_{15}O_5N$, m.p. 209–210°.

The structure of tetrahydroaureothamine[4]

Aureothin (I) and isoaureothin (III) both contain an asymmetric carbon atom, and are thus optically active. The tetrahydro derivative resulting from catalytic hydrogenation may accordingly be a mixture of several stereoisomers. Besides tetrahydroaureothamine (VIII), a considerable amount of an oil which can be isolated in a crystalline state only after conversion to a benzoyl derivative or a tartaric acid salt is



VIII

produced in the case of aureothin (I). The results with isoaureothin (III) were also rather complicated.

Reduction with complex metal hydride[17,18]

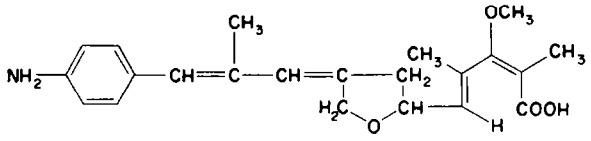
Reduction of isoaureothamine (VII) with lithium borohydride in tetrahydrofuran yielded an acidic substance, aminol H (XVI), $C_{22}H_{27}O_4N$, m.p. 156–158°. Since the carbonyl and methoxyl groups are still present in this compound, it follows that the acidic group should have originated from the reduction with the hydride reagent. An N,O-diacetyl derivative was obtained by acetylation of XVI under non-aqueous conditions (presence of water yielded N-acetyl aminol H). The infra-red band of N,O-diacetyl aminol H (1795 cm^{-1}, α,β-unsaturated acid anhydride, in Nujol) suggested that the acidity (p*K*a 6·1 in 50% aqueous ethanol) of XVI is due to a carboxyl group; a *p*-bromophenacyl ester, m.p. 85–87°, was obtained.

Aminol H (XVI) is still optically active, and the molecular formula shows an increase of two hydrogen atoms when compared with isoaureothamine (VII). Thus, structure (XVI) in which the α-pyrone ring of isoaureothamine has undergone a reductive cleavage is conceivable for aminol H.[19]

[17] K. Yamada, Y. Hirata, K. Okuhara, H. Nakata, T. Naito and K. Iwadare, *J. Chem. Soc. Japan* **79**, 384 (1958).

[18] K. Yamada, T. Naito, K. Okuhara, H. Nakata and Y. Hirata, *Bull. Chem. Soc. Japan*, **33**, 1303 (1960).

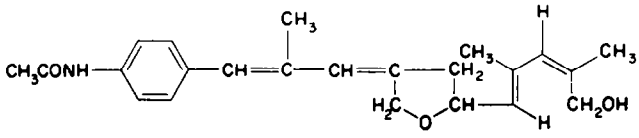
[19] As to the reduction with complex metal hydrides, the investigation, using simple α-pyrones, was carried out in our laboratory, and it was definitely established that a carboxylic acid like aminol H was invariably produced in each case.



XVI

In agreement with this formulation, the derivatives of aminol H show no infra-red absorption around 1575 cm^{-1} attributed to the α-pyrone ring. The structure is also consistent with the fact that more than one mole of methyl pyruvate is obtained by the ozonolysis of its O-methyl derivative (methyl ester).

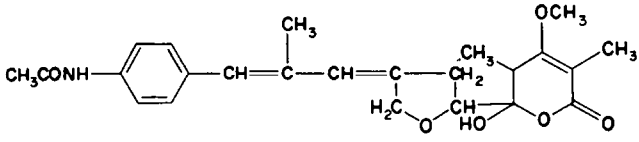
Reduction of N-acetyl O-methyl aminol H with lithium aluminium hydride in tetrahydrofuran results in the loss of the two methoxyl groups present in the starting material to give N-acetyl aminol A (XVII), $C_{23}H_{29}O_3N$, m.p. 155–156°.



XVII

Alkali-catalysed hydration of pyrone ring[18]

When N-acetyl isoaureothamine is heated in 3 N alkali, an acidic substance, was obtained lactol (XVIII), $C_{24}H_{29}O_6N$, m.p. 170–171°, optically inactive. Subtraction of the ultra-violet spectrum of the compound (12) of Table 1 from that of the lactol gave a curve, which showed a maximum at 248 mμ, indicating the destruction of the α-pyrone chromophore in XVIII. Treatment of this compound with acetic anhydride yielded a racemate of the original N-acetyl isoaureothamine. Examination of the infra-red spectra of XVIII and its sodium salt leads to the following structure:



XVIII

Other related natural products

Finally, we wish to emphasise the structural peculiarity of aureothin (I). The compound has a nitro group which is as yet a rather rare function in natural products. Only few other examples are known, including chloramphenicol,[20] hiptagenic acid,[21] azomycin,[22] and aristolochic acid.[23] Moreover, aureothin (I) has the substituted 2-methoxy-γ-pyrone structure. All other methylated 2,4-pyronones which have so far

[20] M. C. Rebstock, H. M. Crooks, Jr., J. Controulis and Q. R. Bartz, *J. Amer. Chem. Soc.* **71**, 2458 (1949).
[21] C. L. Carter and W. J. McChesney, *Nature, Lond.* **164**, 575 (1949).
[22] S. Nakamura, *Pharm. Bull.* **3**, 379 (1955).
[23] M. Pailer, L. Belohlav and E. Simonitsh, *Monatsh.* **87**, 249 (1956).

been found in nature are 4-methoxy-α-pyrones, such as yangonin,[24–28] anibine,[29,30] 4-methoxyparacotoin[29,31] and 5,6-dehydrokavain.[31]

EXPERIMENTAL

Melting points are uncorrected. Ultraviolet spectra were measured in ethanol unless stated otherwise.

Aureothin (I)

Yellow prisms, m.p. 158°. (Found: C, 66·85; H, 5·93; N, 3·43. $C_{22}H_{23}O_6N$ requires: C 66·49; H, 5·83; N, 3·52%). O–CH_3; 7·42% (0·95 mole). C—CH_3; 9·70% (2·57 moles), $=C-\underset{\displaystyle CH_3}{\underset{|}{C}}=$; 21·98% (2·17 moles). $[\alpha]_D$ +51° ($CHCl_3$).

Desmethylisoaureothin (II)

(a) To a hot solution of aureothin (2 g) in ethanol (30 ml), was added 5 ml of conc HCl. After refluxing for 15 min, yellow crystals were formed. The reaction mixture was cooled, the crystals (1·8 g) were collected and washed with cold ethanol. Recrystallization from alcohol gave yellow needles of m.p. 196–198°.

(b) A suspension of aureothin (2·0 g) in 10% HCl (35 ml) was heated for 8 hr on a water bath, when the yellow colour of the crystals was changed to orange. The crystals were collected, washed with water and recrystallized from ethanol giving yellow needles (1·5 g), $[\alpha]_D^{16}$ +118° (ethanol). λ_{max} 248 mμ (log ε 4·26), 346 mμ (log ε 4·30). (Found: C, 65·72; H, 5·32; N, 3·70. $C_{21}H_{21}O_6N$ requires: C, 65·78; H, 5·52; N, 3·65%). C—CH_3; 10·58% (2·71 moles), $=C-\underset{\displaystyle CH_3}{\underset{|}{C}}=$; 20·24% (1·94 moles).

In the usual acetylation with acetic anhydride and pyridine, an *O-acetyl derivative* of II was obtained as yellow needles (IV), m.p. 182–184 (recrystallization from ethanol). λ_{max} 248 mμ (log ε 4·14), 335 mμ (log ε 4·23). (Found: C, 64·96; H, 5·46; N, 3·42. $C_{23}H_{23}O_7N$ requires: C, 64·93; H, 5·45; N, 3·29%).

Isoaureothin (III)

To 1 g of II, 40 ml of ethereal diazomethane solution prepared from 5 g of nitrosomethylurea was added and the mixture was kept at room temp. overnight. The crystals (0·9 g) were collected, washed with a small amount of ether and recrystallized from alcohol. The m.p. was 148–149°, $[\alpha]_D^{18}$ −178° ($CHCl_3$). λ_{max} 250 mμ (log ε 4·17), 335 mμ (log ε 4·19). (Found: 66·71; H, 5·93; N, 3·28. $C_{22}H_{23}O_6N$ requires: C, 66·49; H, 5·83; N, 3·52%). C—CH_3; 9·52% (2·52 moles).

Oxidation with potassium permanganate

(a) To a stirred solution of II (0·5 g) in 0·5 N aq KOH (30 ml), was added a solution of 1·6% aq $KMnO_4$ at room temp under nitrogen till the purple color of permanganate was retained (65 ml, 5 moles). Carbon dioxide was identified. After filtration, the filtrate was acidified and extracted with ether. The ether solution was extracted with 0·1 N NaOH (10 ml). Neutralization of the alkaline solution gave a pale yellow precipitate which was purified by sublimation (180–200°) under reduced pressure, to give white crystals (50 mg), m.p. 234–235° (sealed tube). Methylation with diazomethane and recrystallization from methanol gave colourless crystals of the methyl ester, m.p. 92–93°, undepressed on admixture with an authentic sample of methyl *p*-nitrobenzoate. Oxidation with one mole of $KMnO_4$ gave only *p*-nitrobenzoic acid and the starting material II.

(b) To 2·9 g of I a solution of 3·2% aq $KMnO_4$ (50 ml, 1 mole) was added and the mixture was

[24] W. Borsche and C. K. Bodenstein, *Ber.* **62**, 2515 (1929).
[25] I. Chmielewska and J. Cieslak, *Roczniki Chem.* **28**, 38 (1954).
[26] I. Chmielewska, J. Cieslak, K. Gorczynska, B. Kontnik and K. Pitakowska, *Tetrahedron* **4**, 36 (1958).
[27] J. Cieslak, *Roczniki Chem.* **32**, 837 (1958).
[28] J. D. Bu'Lock and H. G. Smith, *J. Chem. Soc.* 502 (1960).
[29] W. B. Mors, O. R. Gottlieb and C. Djerassi, *J. Amer. Chem. Soc.* **79**, 4507 (1957).
[30] E. Ziegler and E. Nolken, *Monatsh.* **89**, 391 (1958).
[31] O. R. Gottlieb and W. B. Mors, *J. Org. Chem.* **24**, 17 (1959).

stirred for 24 hr at room temp under nitrogen. Carbon dioxide was identified as $BaCO_3$. After filtration of MnO_2 and the starting material, the filtrate was treated in the same manner as described in the case of (a), giving 70 mg of *p*-nitrobenzoic acid.

Chromic acid oxidation

To a solution of I (1·2 g) in glacial acetic acid a solution of chromic acid (150 mg) in glacial acetic acid was added at room temp. The mixture was diluted with twice its volume of water, extracted with chloroform and then the extract was washed with aqueous sodium carbonate. The solvent was removed under reduced pressure and the residue was chromatographed on alumina in benzene solution. Removal of the solvent from the fraction eluted with benzene furnished pale yellow needles, m.p. 108–110°. Recrystallization from methanol gave crystals of m.p. 111–113°, which showed no m.p. depression with synthetic *p*-nitro-α-methylcinnamaldehyde. Elution with benzene–acetone gave the starting material I (300 mg).

Ozonolysis of aureothin

Ozone was bubbled through a cooled solution of 1·0 g of aureothin in 15 ml of chloroform till the yellow colour disappeared (about 2 hr). The solvent was removed under reduced pressure, 5 ml of water was added to the ozonide, and CO_2-free air was bubbled through the mixture kept at 60° for about 2–3 hr. A relatively large amount of carbon dioxide was absorbed by aqueous barium hydroxide solution. From the aqueous layer, formaldehyde and methyl pyruvate were obtained as 2,4-dinitrophenylhydrazone, each of which was obtained by chromatographic separation (For further details concerning the formaldehyde and methyl pyruvate, see the heading on ozonolysis of aureothin and its derivatives). The oily material, separated from aqueous layer, was extracted with ether or benzene. On removal of the solvent under reduced pressure, a crystalline residue was obtained. Recrystallization from ethanol gave colourless needles, undepressed on admixture with the authentic sample of *p*-nitro-α-methylcinnamaldehyde.

Synthesis of p-*nitro-α-methylcinnamaldehyde*

Propionaldehyde (2·2 g, 2·7 ml) was added to a solution of *p*-nitrobenzaldehyde (5·5 g) in 50% aq ethanol (220 ml) and to this there was added dropwise, under stirring, an aqueous solution of sodium hydroxide (0·4 g in 10 ml of water) at room temp. After 30 min with occasional shaking, the precipitated crystalline product (m.p. 107–109°, yield about 5 g) was collected. Recrystallization from absolute alcohol raised the melting point to 112–114°, λ_{max} 297 mμ (log ε 4·30), (Found: C, 63·17; H, 4·91; N, 5·57. $C_{10}H_9O_3N$ requires: C, 62·82; H, 4·75; N, 7·33%).

Reduction of aureothin with $Fe(OH)_2$

A hot solution of 2·0 g of aureothin in 150 ml of alcohol was poured into a hot mixture of 20 g of $FeSO_4 \cdot 7H_2O$, 150 ml of water and 150 ml of conc aqueous ammonia, and the mixture was heated under reflux; the reaction mixture was left to cool at about 40°, and then extracted four times with 50 ml ether. The ethereal extract was dried over K_2CO_3 for a short time and concentrated under reduced pressure to give nearly colourless crystals of V (1·1 g), m.p. 128–129°. Recrystallization from ethanol raised the melting point to 130–131°, C—CH_3; 10·2% (2·5 moles), λ_{max} 241 mμ (log ε 4·24), 309 mμ (log ε 4·41), (Found: C, 72·11; H, 6·86; N, 3·91. $C_{22}H_{25}O_4N$ requires: C, 71·91; H, 6·86; N, 3·81%).

Acetylation of V in the usual way gave an N-*acetyl derivative*, m.p. 166–168°. Treatment with *p*-phenylazobenzoyl chloride under reflux in pyridine, gave yellow crystals, m.p. 227–229°.

Reduction of desmethylisoaureothin with $Fe(OH)_2$

To a hot mixture of $FeSO_4 \cdot 7H_2O$ (15 g), water (80 ml) and conc aqueous ammonia (80 ml), a hot solution of desmethylisoaureothin (1·0 g) in ethanol (100 ml) was added. The black ferric precipitate was filtered and the filtrate was acidified (pH, 4·5) with HCl and thus *desmethylisoaureothamine* (VI) was obtained in the form of its HCl-salt as a white precipitate (910 mg).

The diacetyl derivative of VI was obtained by the direct acetylation of the HCl salt of desmethylisoaureothamine with pyridine and acetic anhydride under reflux. Recrystallization from dioxane gave crystals of m.p. 214–215°, λ_{max} 301·5 mμ (log ε 4·62), (Found: C, 68·41; H, 6·31; N, 3·46. $C_{25}H_{27}O_6N$ requires: C, 68·63; H, 6·22; N, 3·20%).

Reduction of isoaureothin with $Fe(OH)_2$

A hot solution of III (4 g) in alcohol (200 ml) was poured into a hot mixture of $FeSO_4 \cdot 7H_2O$ (50 g), water (200 ml) and conc aqueous ammonia (150 ml) with stirring and the mixture was refluxed for 20 min. After cooling to about 40°, the reaction mixture was extracted three times with 150 ml ether. Upon removal of the solvent from this ether extract, under reduced pressure, white crystals (3·5 g) of *isoaureothamine* (VII) were obtained, which were recrystallized from ethanol, m.p. 134–136°, $[\alpha]_D^{20}$ +163° ($CHCl_3$), λ_{max} 305 mμ (log ε 4·55), (Found: C, 72·00; H, 6·90; N, 4·13. $C_{22}H_{25}O_4N$ requires: C, 71·91; H, 6·86; N, 3·81%), C—CH_3; 11·59% (2·84 moles), OCH_3; 8·27% (0·98 mole).

Acetylation of VII with acetic anhydride in pyridine at room temp gave N-acetyl isoaureothamine, m.p. 214–215°, $[\alpha]_D^{28}$ +140° ($CHCl_3$–CH_3OH), (Found: C, 70·25; H, 6·73; N, 3·97. $C_{24}H_{27}O_5N$ requires: C, 70·42; H, 6·65; N, 3·42%).

Reduction with aluminium amalgam

To a solution of 5 g of aureothin in 300 ml ethanol, aluminium amalgam and a small amount of water was added, and the mixture was stirred for 24 hr. Removal of the solvent under reduced pressure from the filtrate and hot alcoholic washings gave a product, which was extracted with ether. The ethereal solution was purified by chromatography on alumina and the eluted portion was evaporated to furnish crystals. Recrystallization from alcohol gave colourless crystals, m.p. 127–129°, undepressed on admixture with an authentic specimen of V.

Clemmensen reduction

A mixture of I (0·35 g), ethanol (10 ml), benzene (5 ml), amalgamated zinc and conc HCl (3 ml) was refluxed for 4 hr. The resulting white needles (0·2 g), filtered and washed with ethanol, had m.p. 210–215°. This compound was the hydrochloride of desmethylisoaureothamine, because its acetylation gave N,O-diacetyl desmethylisoaureothamine, which was obtained by the acetylation of VI.

Reductive acetylation

A mixture of II (130 mg), acetic anhydride (5 ml), two drops of pyridine and zinc was heated on a water-bath for 5 min. After filtration of the zinc, addition of water (50 ml) to the filtrate gave a white precipitate which gave crystals (110 mg), m.p. 216–218°, undepressed on admixture with an authentic sample of N,O-diacetyl desmethylisoaureothamine.

Isomerization of aureothamine

V (0·1 g), ethanol (5 ml) and conc HCl were heated on a water-bath for 15 min. The resulting white needles (0·06 g) filtered and washed with alcohol, had m.p. 210–214° (dec). Acetylation in the usual way gave N,O-diacetyl desmethylisoaureothamine.

Isomerization of N*-acetyl aureothamine*

To a solution of N-acetyl aureothamine (2·5 g) in ethanol (30 ml) ethanol (4 ml) and conc HCl (4 ml) were added, and the mixture was heated on a water-bath for 5 min. The resulting precipitate was filtered and crystallized from ethanol to give 2·0 g of colourless crystals, m.p. 217–220° (dec). Acetylation in the usual way gave N,O-diacetyl desmethylisoaureothamine.

Catalytic hydrogenation of aureothin

A suspension of 5·0 g of aureothin in 150 ml of warm alcohol was hydrogenated at room temp under atmospheric pressure of hydrogen in the presence of 500 mg of platinum oxide; after absorption of about 5 moles (ca. 1600–1750 ml) of hydrogen, the hydrogenation had practically ceased. The almost colourless filtrates were concentrated under reduced pressure in a stream of nitrogen and to the resultant oily material 10 ml of ether was added and it was allowed to stand for several days in an ice box. White crystals of *tetrahydroaureothamine* (VIII) were collected, and recrystallization from benzene gave colourless plates, m.p. 130–131°, λ_{max} 243 mμ (log ε 4·24), a shoulder at 276 mμ (log ε 3·94), (Found: C, 71·13; H, 7·90; N, 4·29. $C_{22}H_{29}O_4N$ requires: C, 71·13; H, 7·83; N, 3·77%), C—CH_3; 2·13 moles, $[\alpha]_D^{16}$ +36° ($CHCl_3$). The crystallized tetrahydroaureothmine was used for the preparation of benzoate A (m.p. 171–172°), benzoate B (m.p. 167–168°) and the residual

oily material was used for the preparation of benzoate C (m.p. 190–191°). Benzoate B was changed to benzoate D (m.p. 187–188°) on heating at 170°.

N-Benzoyl tetrahydroaureothamine (benzoate A)

To a solution of 600 mg of VIII in 7 ml of pyridine 300 mg of benzoyl chloride was added and the mixture was warmed at 80° for 10 min. The mixture was cooled below 0° and a small amount of crushed ice was added under cooling. The mixture was then poured into 50 ml of water and the precipitated white solid was collected and washed with water thoroughly (not with any dilute acid) and with a dilute solution of sodium carbonate and again with water, and recrystallized twice from ethanol; colourless or slight yellow pyramids, m.p. 171–172°, λ_{max} 271 mμ (log ε 4·17), (Found: C, 73·39; H, 7·11; N, 3·05. $C_{29}H_{33}O_5N$ requires: C, 73·24; H, 7·00; N, 2·95%), OCH_3; 6·53%. The substance gave a negative test for an aromatic amino group with *p*-N,N-dimethylaminobenzaldehyde, with ninhydrin and with 1,2-naphthoquinone-4-sulphonic acid.

When the same amine VIII was subjected to benzoylation under slightly more vigorous conditions than those employed in the preparation of benzoate A, the product having m.p. 167–168° (*benzoate B*) was obtained.

Catalytic hydrogenation of isoaureothin

Isoaureothin (3 g) was suspended in 180 ml of ethanol containing 300 mg of platinum oxide and hydrogenated at atmospheric pressure and room temp. The consumption of hydrogen ceased after 1100 ml (5·1 moles) had been taken up. The catalyst was separated by filtration and the colourless filtrate was concentrated under reduced pressure when an oily material was obtained. This oily product was kept in an ice box for a few weeks but no crystalline substance appeared. The oily product in ether-benzene was chromatographed on alumina, and concentration of the eluted fraction with benzene gave 2 g of a slightly yellow oily material.

A solution of 2 g of this oily material in 10 ml of pyridine was treated with 1 g of benzoyl chloride and kept at 80° for 20 min. After cooling the solution, it was poured onto 20 g of crushed ice. On allowing the resultant red-brown oily material to stand overnight at room temp, a crystalline substance appeared in the solidified mixture. Recrystallization from ethanol twice gave white needles of *N-benzoyl tetrahydroisoaureothamine* (600 mg), m.p. 165–166°, λ_{max} 280 mμ (log ε 4·27), $[\alpha]_D^{25}$ 0° (methanol), (Found: C, 73·24; H, 7·04. $C_{29}H_{33}O_5N$ requires: C, 73·24; H, 7·00%). Infra-red spectrum, 1692 (C=O and amide I), 1592, 1577, 1525 (sh), 1513 cm^{-1} (KBr). The optical inactivity does not mean that the compound was racemized, since the ozonolysis of this compound gave N-benzoyl aminotetrahydroaureothinic acid (IX) which had an optical activity. The concentration of mother liquor of crystalline N-benzoyl tetrahydroisoaureothamine led to a large amount of oily material, the infra-red spectrum of which was identical with that of the crystals.

Catalytic hydrogenation of desmethylisoaureothin

Desmethylisoaureothin was hydrogenated and the resultant tetrahydrodesmethylisoaureothin was benzoylated to give *benzoate E* (m.p. 167–168°) whose infrared spectrum was almost identical to those of benzoate B, C and D. Benzoate E was changed to benzoate F on heating at 107°. Recrystallization from alcohol gave colourless needles, m.p. 187–188°.

Synthetic model compounds for ultra-violet spectroscopic study

Syntheses of model compounds listed in Table 1 are described here.

(a) *5-(p-Nitrophenyl)-4-methyl-2,4-pentadien-1-al.* To a solution of *p*-nitro-α-methylcinnamaldehyde (1 g, whose synthesis has been described in another part of this paper) and acetaldehyde (1 ml) in ethanol (50 ml), was added dropwise a 0·5% NaOH aq solution (30 ml) with vigorous stirring at room temp over a period of 20 min. The bright yellow precipitate was collected and recrystallized several times from alcohol to yield leaflets, m.p. 141–143°, (Found: C, 66·62; H, 5·10; N, 6·01. $C_{12}H_{11}O_3N$ requires: C, 66·35; H, 5·09; N, 6·45%).

(b) *6-(p-Nitrophenyl)-5-methyl-3,5-hexadien-2-one.* To a chilled solution of *p*-nitro-α-methylcinnamaldehyde (1 g) in acetone (30 ml), under stirring, 5 ml of 0·5% aq NaOH was added dropwise, when the solution became dark-coloured. After about 20 min at room temp, this was acidified with 5 ml of 1:3 aq HCl to give a bright yellow solution. A few minutes later, the solution was diluted

with water (60 ml), when a yellow crystalline precipitate appeared; this was recrystallized repeatedly from ethanol to give yellow needles (350 mg), m.p. 116–118°, (Found: C, 67·72; H, 5·87; N, 6·04. $C_{13}H_{13}O_3N$: requires: C, 67·52; H, 5·67; N, 6·08%).

(c) 8-(p-*Nitrophenyl*)-7-*methyl*-3,5,7-*octatrien*-2-*one*. This was synthesized by condensation of 5-(*p*-nitrophenyl)-4-methyl-2,4-pentadien-1-al with acetone under the same conditions as described in the case of (a), orange yellow crystal, m.p. 135–137°, (Found: C, 70·09; H, 5·95; N, 5·43. $C_{15}H_{15}O_3N$ requires: C, 70·02; H, 5·88; N, 5·44%).

(d) p-*Amino*-α-*methylcinnamaldehyde*. A hot solution of 300 mg of *p*-nitro-α-methylcinnamaldehyde in 15 ml of ethanol was added with stirring to a hot mixture of $FeSO_4 \cdot 7H_2O$ (5 g), water (20 ml) and conc aq ammonia (10 ml). After 10 min at 80°, the reaction mixture was extracted with ether. The ether solution, after drying with K_2CO_3, was evaporated under reduced pressure and crystals were obtained. Recrystallization from ethanol gave orange yellow needles, m.p. 178–180°, (Found: C, 74·59; H, 6·98; N, 8·80. $C_{10}H_{11}ON$ requires: C, 74·51; H, 6·88; N, 8·69%).

(e) 6-(p-*Aminophenyl*)-5-*methyl*-3,5-*hexadien*-2-*one*. The nitro compound, the synthesis of which was described in (b), was treated with $FeSO_4 \cdot 7H_2O$–aq NH_3 in the same manner as described in (d), m.p. 132–133°, (Found: C, 77·51; H, 7·41; N, 7·14. $C_{13}H_{15}ON$ requires: C, 77·58; H, 7·51; N, 6·96%).

(f) p-*Nitro*-α-*methylcinnamyl alcohol*. A solution of 500 mg of *p*-nitro-α-methylcinnamaldehyde in 20 ml of ethanol was treated with $NaBH_4$ (50 mg) in 20 ml of ethanol, and refluxed for 20 min. After cooling, the solution was neutralized (pH 7) with dilute HCl, and extracted with ether. The ether extract was evaporated under reduced pressure and an orange yellow oil remained which crystallized after placing in an ice box, m.p. 46–47°.

(g) 6-(p-*Nitrophenyl*)-5-*methyl*-3,5-*hexadien*-2-*ol*. To a solution of 350 mg of the nitro-ketone compound, described in (b), in tetrahydrofuran (10 ml) 50 ml of $LiBH_4$ was added and the mixture was kept at room temp for 20 min. After addition of water (5 ml), the solvent was removed and a red-brown oil was obtained, which became solid. Upon recrystallization from ethanol, yellow needles appeared (200 mg), m.p. 86–88°, (Found: C, 67·38; H, 6·26; N, 6·41. $C_{13}H_{15}O_3N$ requires: C, 66·93; H, 6·48; N, 6·01%).

(h) p-*Amino*-α-*methylcinnamyl alcohol*. The nitro-alcohol, described in (f) and obtained from 500 mg of *p*-nitro-α-methylcinnamaldehyde, in ethanol (15 ml) was poured into the mixture of $FeSO_4 \cdot 7H_2O$ (5 g), water (15 ml) and conc aq ammonia (10 ml) and the resultant mixture was heated for 15 min. The mixture was filtered and the filtrate was extracted with ether. The ether extract was concentrated under reduced pressure to give crystals. Recrystallization from water gave colourless crystals (80 mg), m.p. 82–83°.

This amino alcohol was treated with acetic acid–acetic anhydride (2:1) at room temp for 10 min and then water was added. The resultant white precipitate was recrystallized from water to give p-*acetylamino*-α-*methylcinnamyl alcohol*, m.p. 123–125°.

(i) 6-(p-*Aminophenyl*)-5-*methyl*-3,5-*hexadien*-2-*ol*. To a solution of 150 mg of the corresponding nitro alcohol described in (g) a mixture of $FeSO_4 \cdot 7H_2O$ (2 g), water (75 ml) and conc aq ammonia (10 ml) was added. The mixture was heated for 10 min and extracted with ether, and the resultant ether extract was evaporated under reduced pressure to give an oily material. Ligroin (30 ml) was added to this oily product and heated. After the insoluble part was removed, the ligroin solution was cooled to give slightly yellow crystals, which upon recrystallization from ligroin gave needles (40 mg), m.p. 84–86°, (Found: C, 76·01; H, 8·13; N, 6·76. $C_{13}H_{17}O\,N$ requires: C, 76·81; H, 8·43; N, 6·89%).

Acetylation of this compound under the same condition shown in the case of (h) gave N-*acetyl derivative*, m.p. 123–125°.

Analysis of C—CH_3

The determination of the C—CH_3 group was carried out by the Kuhn–Roth method. On distilling the mixture three or four times, the volatile acids produced on oxidation, were almost entirely in the distillates. In the case of the *p*-nitroaromatic compound, however, the distillate, obtained after the repeated distillation (nine times) of the oxidized product, was still acidic and consumed a small amount of alkali. This was due to the fact that *p*-nitrobenzoic acid, produced by oxidation, was gradually distilled out. This was confirmed by an experiment using authentic *p*-nitrobenzoic acid.

In the present case, the distillations were repeated seven times, and the amount of alkali consumption observed was corrected for the co-existence of *p*-nitrobenzoic acid in the distillates, on the basis of the following data.

TABLE 4. CONSUMPTION OF ALKALI DUE TO *p*-NITROBENZOIC ACID (DISTILLATIONS WERE REPEATED SEVEN TIMES)

p-Nitrobenzoic acid (mg)	Consumption of alkali (ml) N/100 NaOH (f 1·07)	Consumption of alkali per 1 mg *p*-nitrobenzoic acid (ml)	
		obs.	mean
4·069	0·572	0·145	
1·853	0·362	0·195	0·155
4·475	0·557	0·124	

Ozonolysis of aureothin and its derivatives

Ozonolysis of aureothin and its derivatives was carried out as follows.

Ozone was bubbled through the cooled chloroform solution for about 2–3 hr, until the absorption of ozone practically ceased. The solvent was removed and the resultant ozonide was decomposed by warming at 60° with 10 ml of water. Carbon dioxide was trapped by aqueous barium hydroxide solution (0·5–1 mole as $BaCO_3$), and a small amount of volatile carbonyl compounds was caught by a 2 N HCl solution of 2,4-dinitrophenylhydrazine. An oily product was separated from the aqueous layer and washed with water several times. This oily product soon solidified and was recrystallized from alcohol to give crystals of *p*-nitro-α-methylcinnamaldehyde (*vide supra*).

Addition of a 2,4-dinitrophenylhydrazine solution (2 N HCl) to the combined solution of the aqueous layer and the washings gave a mixture of hydrazones. The dried mixture of the hydrazones was extracted with hot benzene, and the benzene solution was chromatographed on alumina to separate each component. Sometimes by cooling the benzene extract, crystals (most of them were the 2,4-dinitrophenylhydrazone of methyl pyruvate) were separated. (In the case where ozonization had given formaldehyde, 2,4-dinitrophenylhydrazone of formaldehyde was eluted as the first fraction by chromatography on alumina. The solvent was removed and the residue was recrystallized from alcohol to give yellow needles, m.p. 156–158°, undepressed on admixture with authentic sample of 2,4-dinitrophenylhydrazone of formaldehyde.)

There were two kinds of 2,4-dinitrophenylhydrazones of methyl pyruvate and both of them were obtained by ozonolysis. These two hydrazones were separated by chromatographic techniques as a yellow band and a brown band.

The two kinds of 2,4-dinitrophenylhydrazones are as follows.

Form I: yellow orange needles, m.p. 179–180° (To methanol containing H_2SO_4, pyruvic acid and 2,4-dinitrophenylhydrazine was added and then the mixture was heated).

Form II: yellow prisms, m.p. 147–148° (2,4-dinitrophenylhydrazine solution was added to methyl pyruvate).

The yields of the products from various derivatives are listed in Table 5.

Ozonolysis of N-*benzoyl tetrahydroaureothamine*

Through a cooled solution (0°–10°) of 515 mg of N-benzoyl tetrahydroaureothamine in 10 ml of chloroform ozone was bubbled until about two equivalents of ozone were consumed (which was determined by techniques modified from gas analysis). After the removal of the solvent under reduced pressure, the ozonide was decomposed by adding 10 ml of water, and CO_2-free nitrogen gas was bubbled through the warmed mixture (60°) for 1 hr. The aqueous layer was separated from the oily material and treatment of this aqueous layer with 2,4-dinitrophenylhydrazine solution gave about 40 mg of hydrazone mixture, from which two kinds of yellow hydrazones (A and B, for convenience) were separated by chromatographic techniques. "A" was again chromatographed on alumina and was characterized as the form II of 2,4-dinitrophenylhydrazone of methyl pyruvate,

yellow needles, m.p. 179–180°, undepressed by admixture with an authentic sample. Another B was recrystallized from ligroin–benzene (5:1), bright yellow prisms, m.p. 144–145°, undepressed on admixture with an authentic sample of form I of 2,4-dinitrophenylhydrazone of methyl pyruvate.

TABLE 5. YIELDS OF THE PRODUCTS BY OZONOLYSIS

Compound	Formaldehyde (equiv. mole)	Methyl pyruvate (equiv. mole)
Aureothin	0·19	0·01–0·02
Isoaureothin	0·12	0·19
N-Acetyl tetrahydroaureothamine	—	trace
N-Acetyl tetrahydroisoaureothamine	—	ca. 0·1
N-Benzoyl tetrahydroisoaureothamine	—	0·15
N-Acetyl isoaureothamine	trace	0·21
N-Acetyl aminol H	—	0·22
N-Acetyl O-methyl aminol H	—	1·05

The fraction (oil) insoluble in water was dissolved in ether which was shaken with conc aq $NaHCO_3$ solution. After the sodium bicarbonate solution was left for some time, white crystals precipitated, the sodium salt of N-benzoyl amino tetrahydroaureothinic acid (IX), 120 mg. The ether layer was washed with water several times, dried with anhydrous sodium sulphate and concentrated. The resultant colourless oily substance which was chromatographed on alumina in ethereal solution showed an infra-red band of γ-lactone at 1772 cm^{-1} and was shown to be identical with the neutral oily product obtained by the ozonolysis of N-benzoyl tetrahydroisoaureothamine, λ_{max} 272 mμ (*cf.* N-benzoyl *p*-toluidine, λ_{max} 271 mμ), $[\alpha]_D^{26}$ +39 ± 3° (CH_3OH).

N-*Benzoyl aminotetrahydroaureothinic acid* (IX)

The sodium salt, thus obtained was rather insoluble in water and soluble in methanol and ethanol, infra-red spectrum, 1598 cm^{-1} (KBr, carboxylate).

For the purpose of the isolation of free IX, an aqueous methanol solution of the sodium salt was acidified with dil HCl, giving only an oily material. However, by adding water to the solution of the sodium salt in pyridine–acetic anhydride, colourless crystals were obtained, m.p. 83–86°. The methanolic solution containing 30 mg of sodium salt of IX and 50 mg of *p*-bromophenacyl bromide was refluxed for 1 hr. Water was added to the warm solution until the solution became slightly turbid. After cooling, 25 mg of *p-bromophenacyl ester* of IX precipitated gradually. This was collected and dried. Recrystallization from ethanol–water gave plates, m.p. 145–146°, (Found: C, 63·59; H, 5·43; N, 2·41; Br, 14·20. $C_{30}H_{30}O_8NBr$ requires: C, 63·83; H, 5·36; N, 2·48; Br, 14·17%), C—CH_3, 2·16% (0·87 mole), $[\alpha]_D^{28}$ +23° (CH_3OH).

The ozonolysis of N-benzoyl tetrahydroisoaureothamine by the same method gave a sodium salt. The *p*-bromophenacyl ester had m.p. 145–146°, undepressed by admixture with the ester of IX.

Synthesis of α-alkoxy carboxylic acid and ester

These compounds were synthesized to use as model compounds for infra-red spectra. The details have been reported.[12]

Aureothinic acid (X)

To a stirred solution of 2 g of I in 50 ml ethanol, 2 g of KOH in 10 ml ethanol and hydrogen peroxide (30%), were added alternately.

The yellow solution turned brown on addition of excess KOH solution, and became yellow again on addition of hydrogen peroxide solution. The mixture was kept orange during the reaction by adjusting the addition of the reagents. The addition of hydrogen peroxide was continued till the crystals of I disappeared and a white inorganic substance appeared. About 1·5–2·0 g of KOH and 5–6 ml of hydrogen peroxide (30%) were added for 8 hr. Excess water was added to the reaction mixture which became clear. The solution was acidified with 6 N HCl. The acidic aqueous solution

was extracted with ether three times. The ether extract was shaken with conc sodium bicarbonate solution four times, when aureothinic acid (X) was extracted with sodium bicarbonate solution and a neutral substance, *aureothinic ketone* (XI) remained in the ether layer. The sodium bicarbonate solution was acidified, and then extracted with ether three times. The ethereal layer thus obtained, after shaking with water, was concentrated under reduced pressure. The residue was cooled in the refrigerator and the slightly yellow crystals were filtered and recrystallization from ethanol gave the crystals of X, m.p. 149–151°, yield 0·2 g, λ_{max} 249 mμ (log ε 4·14), 345 mμ (log ε 4·20), $[\alpha]_D^{28}$ +54° (CH_3OH), (Found: C, 62·31; H, 5·29; N, 4·89. $C_{15}H_{15}O_5N$ requires: C, 62·28; H, 5·23; N, 4·84%), C—CH_3, 4·70% (0·91 mole), infra-red spectrum, 1760, 1722, 1595, 1511 cm^{-1} ($CHCl_3$).

The ether layer, which contained ketone XI, was dried with anhydrous sodium sulphate, chromatographed on alumina, and the second eluted fraction was collected. Removal of the solvent gave ketone XI, which was recrystallized from alcohol as yellow needles (10–20 mg), m.p. 103–105°, λ_{max} 248 mμ (log ε 4·20), 342 mμ (log ε 4·28), $[\alpha]_D^{21}$ +16·3°, (Found: C, 67·38; H, 6·49; N, 4·75. $C_{17}H_{19}O_4N$ requires: C, 67·76; H, 6·39; N, 4·65%), C—CH_3, 9·40% (1·88 moles).

Ozonolysis of aureothinic acid

A solution of 500 mg (1·74 mmoles) of X in 15 ml of chloroform was cooled to about −5° and ozone was passed through this solution till one equivalent amount (1·74 mmoles) of ozone was absorbed. The solution became almost colourless. The solvent was removed under reduced pressure at room temp and the resulting crystalline ozonide was decomposed in a flask fitted with a reflux condenser, by warming at 60° with 10 ml of water. The resultant oily material, insoluble in water, crystallized after cooling. The crystals were *p*-nitro-α-methylcinnamaldehyde as shown by infra-red measurement. The colorless aqueous layer obtained from the decomposition of the ozonide was distilled under reduced pressure into a 2,4-dinitrophenylhydrazine solution. From this solution, about 70 mg of hydrazone was obtained, which was chromatographed on alumina; this was identified as the 2,4-dinitrophenylhydrazone of formaldehyde, m.p. 156–158°, undepressed by admixture with an authentic sample. The unvolatile fraction remaining in the flask was a mixture of oily and crystalline materials and was acidic. The colour reaction for 1,2-dicarboxylic acids was positive. This acid (50 mg) was neutralized with sodium bicarbonate (50 mg) and treatment of this sodium salt with *p*-bromophenacyl bromide (280 mg) in ethanol–water yielded a crystalline substance which was recrystallized from acetone; this was the *p*-bromophenacyl ester of malic acid, m.p. 175–177°, confirmed by a mixed melting point test and comparison of infra-red spectrum with that of an authentic specimen.

Chromic acid oxidation of aureothinic ketone

By the Kuhn–Roth method, 10 mg of XI was oxidized and the product was distilled. To the distillate (50 ml) conc aqueous ammonia (5 ml) was added, and it was evaporated to dryness under diminished pressure. The residue was dissolved in a drop of water, and propionic acid and acetic acid were identified by a paper chromatographic method.

Alkaline degradation of aureothin

To a solution of I (2 g) in ethanol (16 ml) a solution of KOH (2 g) in water (7 ml) was added and the mixture was heated at 80° for 2 hr under nitrogen. Volatile carbonyl compounds were trapped by 2,4-dinitrophenylhydrazine solution and thus 10 mg of 2,4-dinitrophenylhydrazone was collected. The filtrate of the reaction mixture was neutralized with HCl, when a fairly large amount of precipitate appeared. After the filtration, a solution of 2,4-dinitrophenylhydrazine was added to this filtrate to give 10 mg of 2,4-dinitrophenylhydrazone. Recrystallization from alcohol gave crystals of m.p. 148–150°, undepressed on admixture with an authentic sample of 2,4-dinitrophenylhydrazone of diethyl ketone.

Alkaline degradation of isoaureothin

By the same method as described above, diethyl ketone was obtained from isoaureothin as 2,4-dinitrophenylhydrazone.

Alkaline degradation of desmethylisoaureothin

The starting material was recovered, even when somewhat drastic conditions were employed as compared with those in the case of aureothin.

Aureonone (XII)

A solution of I (3 g) in 30 ml of chloroform was cooled to about $-5° \sim -10°$ and ozone was passed through this solution till one equivalent amount of ozone was absorbed. The solvent was removed under reduced pressure at room temp and the resulting crystalline ozonide was decomposed in a flask fitted with reflux condenser, by warming at 60° with 10 ml of water for 1 hr. After cooling, the separated solid was treated with 10–15 ml of water at 80° five times. The water insoluble residue was mainly *p*-nitro-α-methylcinnamaldehyde. The combined solution of the filtrate and washings was distilled under reduced pressure into a solution of 2,4-dinitrophenylhydrazine to give 57 mg of yellow precipitate which was identified as 2,4-dinitrophenylhydrazone of formaldehyde by recrystallization. The unvolatile fraction remaining in the flask was an oily material (but partly crystalline), to which was added a small amount of methanol and on keeping in an ice box gave aureonone (XII) as prisms. The crystals were filtered and washed with ether–methanol. Removal of the solvent from the filtrate gave additional crystals, overall yield 180 mg. Recrystallization from a small amount of methanol gave crystals, m.p. 153–155°, λ_{max} 263 mμ (log ε 3·90), 248 mμ (log ε 3·84), (Found: C, 60·28; H, 6·01. $C_{12}H_{14}O_5$ requires: C, 60·50; H, 5·92%).

Isomerization of aureonone

On heating at 40° in ethanol containing a small amount of HCl, aureonone was easily isomerized. This was identified by the changes in its ultra-violet spectrum.

Isoaureonone (XIII)

A solution of 0·5 g of isoaureothin (III) in 15 ml of chloroform was cooled to $-5° \sim -10°$ and ozone was passed through this solution till 1·1 equivalent amount of ozone was absorbed. By the same method as described above, the ozonide of isoaureothin was obtained and then decomposed at 50° with 10 ml of water. After cooling, the separated solid was treated with 10 ml of hot water five times. The water insoluble residue was crystallized easily and was proved to be *p*-nitro-α-methylcinnamaldehyde. A combined solution of filtrate and washings was distilled under reduced pressure into a solution of 2,4-dinitrophenylhydrazine, to give 15 mg of yellow precipitate. The unvolatile residue was pale yellow oil. In spite of repeated purification by various methods, crystallization of this unvolatile substance (isoaureonone) did not take place, λ_{max} 294 mμ.

Methylation of desmethylisoaureothin with diazomethane

To a suspension of 870 mg of II in 30 ml of dry ether 50 ml of ethereal diazomethane solution, prepared from 1 g of nitrosomethylurea, was added dropwise over a period of 18 hr with vigorous stirring. During this period, the temp of the reaction mixture was maintained at 10–15° and traces of atmospheric moisture were excluded. After the diazomethane solution had been added, stirring was continued for 2 hr. The yellow crystalline solid was then filtered and washed with three 10 ml portions of ether. The ether washings were combined with the filtrate. The solid which separated was recrystallized from ethanol and 620 mg of isoaureothin (III) was obtained, m.p. 146–147°, undepressed on admixture with an authentic sample. Since aureothin does not form a hydrochloride in spite of the presence of the γ-pyrone structure, two isomeric methyl ethers, I and III, were separated by fractional crystallization from ethanol. Thus, the above ether filtrate and washings were combined and were evaporated to dryness *in vacuo*. Recrystallization of the resulting solid from ethanol gave a mixture of yellow prisms and of a small amount of light yellow needles. Two additional recrystallizations from ethanol gave 10 mg of aureothin (I), yellow prisms, m.p. 152–153°, undepressed on admixture with an authentic sample.

Methylation of desmethylisoaureothin with diemthyl sulphate

(a) II (1 g) was dissolved in 30 ml of hot 2 N NaOH solution. A clear brown solution was obtained. The crystals which separated on cooling were collected, washed with a small amount of cold alcohol

and dried. Light yellow needles, 1·1 g. The salt had no distinct melting point and decomposed at about 207–210° without melting.

(b) Methylation of the sodium salt of II: the sodium salt (1·1 g) was suspended in 40 ml of anhydrous acetone. Dimethyl sulphate (0·7 g) was added to the suspension and the mixture was refluxed for 6 hr, and allowed to stand overnight. The mixture was again warmed on a water bath for 5 min and was filtered. Recrystallization from alcohol twice gave yellow needles, m.p. 146°, undepressed on admixture with an authentic sample of III. yield, 200 mg. Spontaneous evaporation of each mother liquor from recrystallization gave 60 mg of brownish yellow prisms, m.p. 151–152°, which was found to be identical (by mixed m.p. and infra-red spectrum) with I.

The above acetone filtrate was diluted with 30 ml of water. The separated brown oil crystallized after 2 days. Recrystallization from alcohol gave an additional 180 mg of III. The mother liquor was evaporated as above, and 205 mg of I, m.p. 150–151° was obtained as brownish yellow prisms, total yield 62% (I, 265 mg; III, 380 mg). When the reaction mixture was refluxed for 5 hr, 40 mg of I and 110 mg of III was obtained from 230 mg of II (total yield, 63%). In another run, the reaction mixture was refluxed with 2 g of anhydrous K_2CO_3. From 1 g of the sodium salt, 150 mg of I and 250 mg of III were obtained (total yield, 41%). In each case, none of the starting material of II was recovered.

Aza-desmethylisoaureothin (XIV)

A suspension of 1 g of aureothin (I) 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 crystallized on the addition of 50 ml of hot ethanol. After washing three times with hot ethanol, the product was recrystallized from dimethylformamide; weight 250 mg, m.p. 238–239°, λ_{max} 341 mμ (log ε 4·25), 296 mμ (log ε 4·16), 245 mμ (log ε 4·18), (Found: C, 65·76; H, 5·95; N, 7·63. $C_{21}H_{22}O_5N_2$ requires: C, 65·95; H, 5·80; N, 7·33%).

In a solution of 1 ml of pyridine and 5 ml of acetic anhydride 20 mg of XIV was suspended. The mixture became a clear solution when kept at 70–80° 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 *aza-desmethylisoaureothin acetate* with m.p. 211–213° were obtained (15 mg), λ_{max} 325 mμ (log ε 4·32), 238 mμ (log ε 4·30).

Aza-desmethylisoaureonone acetate (XV)

A solution of 350 mg of aza-desmethylisoaureothin acetate in 15 ml of chloroform was cooled to $-5° \sim -10°$ and ozone was passed through this solution, until about one equivalent amount of ozone was absorbed. On removal of the solvent under reduced pressure, a gummy residue was obtained which was treated with 10 ml of water in a flask fitted with a reflux condenser at 60°. The aqueous layer in the flask was separated from the solid residue by decantation and the aqueous fraction was distilled under reduced pressure to give a slightly yellow residue, which on recrystallization from methanol, weighed 10 mg, m.p. 208–209°. 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 infra-red spectrum. The latter, sparingly soluble in methanol, was recrystallized from methanol and gave 70 mg of colourless needles, which turned out to be identical with the crystals obtained from the water soluble fraction, as described above. The total amount was 80 mg, m.p. 209–210°, λ_{max} 304 mμ (log ε 3·85), 233 mμ (log ε 3·81), (Found: C, 58·86; H, 5·98; N, 5·45. $C_{13}H_{15}O_5N$ requires: C, 58·83; H, 5·70; N, 5·28%).

Aminol H (XVI)

When a solution of isoaureothamine (VII) (2 g) and $LiBH_4$ (180 mg) in purified tetrahydrofuran (60 ml) was warmed on a water bath, a reaction started with the generation of gas; about 30 min later, it was refluxed for 20 min, the excess $LiBH_4$ and complex were decomposed with water, and the solvent was removed under reduced pressure. To this residue, on adding water, an almost transparent solution was obtained: the aqueous layer (pH ca. 10) was neutralized to pH 5·5, the

precipitated crystals were collected, and the remaining product was extracted thrice with ether from the mother liquid. The combined reduction product was recrystallized from ethanol, the first crop being 1·2 g; an analytical sample was recrystallized from alcohol repeatedly, m.p. 156–158°, $[\alpha]_D^{27}$ +13° (CH_3OH), λ_{max} 304 mμ (log ε 4·47), (Found: C, 71·62; H, 7·58; N, 3·60. $C_{22}H_{27}O_4N$ requires: C, 71·52; H, 7·37; N, 3·79%), C—CH_3 10·25% (2·52 moles), OCH_3 8·08% (0·96 moles).

Aminol H was treated with diazomethane in ether when it methylated rather easily with the generation of gas. An oily product crystallized out during the course of several days. It was recrystallized from absolute ethanol by addition of petroleum ether, m.p. 92–94°, (*O-methyl aminol* H), (Found: C, 72·07; H, 7·39; N, 3·86. $C_{23}H_{29}O_4N$ requires: C, 72·03; H, 7·62; N, 3·65%), OCH_3, 14·93% (1·85 moles).

Aminol H was warmed in acetic anhydride for a few minutes and after decomposition of the acetic anhydride by pouring into water, an oily product was obtained; it was recrystallized from alcohol–petroleum ether repeatedly, m.p. 129–132°. It was shown to be N-*acetyl aminol H* from analytical and infra-red evidence.

Aminol H was dissolved in a small amount of acetic anhydride–pyridine mixture and the solution was warmed for a few minutes on a water bath. After cooling, the solution was diluted with ether and further petroleum ether was added until the solution became turbid. The solution was allowed to stand in an ice box when crystals appeared. Recrystallization from absolute ethanol–petroleum ether gave fine crystals of N,O-*diacetyl aminol* H, m.p. 94–95°, infra-red spectrum 1795, 1712, 1660, 1511 cm^{-1} (KBr). Treatment of this diacetyl derivative with water, gave N-acetyl aminol H.

N-*Acetyl* O-*methyl aminol* H was obtained in two ways: acetylation of O-methyl aminol H under the usual conditions (acetic anhydride–pyridine) or methylation of N-acetyl aminol H with diazomethane. Recrystallization from ethanol gave needles, m.p. 130–132°, λ_{max} 298 mμ (log ε 4·51), (Found: C, 70·56; H, 7·53; N, 3·43. $C_{25}H_{29}O_4N$ requires: C, 70·56; H, 7·34; N, 3·29%).

N-*Acetyl aminol* A (XVII)

To N-acetyl O-methyl aminol H (190 mg) in tetrahydrofuran (8 ml) lithium aluminium hydride (100 mg) in tetrahydrofuran was added and the mixture was stirred at room temp for 20 min. After addition of ethyl acetate to decompose the excess $LiAlH_4$, water saturated with Na_2SO_4 was added and then the solution was acidified with dilute HCl. The solution was extracted with ether. The ether extract was washed with $NaHCO_3$ solution, dried with Na_2SO_4 and concentrated under reduced pressure to give a yellow solid. Recrystallization from ethanol gave colourless needles of XVII, m.p. 155–156°, $[\alpha]_D^{27}$ +57·6° ($CHCl_3$), λ_{max} 300 mμ (log ε 4·54), (Found: C, 75·32; H, 8·02; N, 3·89. $C_{23}H_{29}O_3N$ requires: C, 75·17; H, 7·95; N, 3·81%).

Treatment of XVII with acetic anhydride–pyridine gave crystals of N,O-*diacetyl aminol* A, m.p. 144–146° (recrystallization from ethanol).

Lactol (XVIII)

To a mixture of 110 ml of ethanol and 55 ml of 3 N aq NaOH, 2 g of N-acetyl isoaureothamine was added. The flask was equipped with a reflux condenser and the suspension was warmed on a water bath at about 80° for 10 min, when the mixture became clear and of an orange colour. After rapid cooling, the solution was neutralized with dilute HCl; the solution turned yellow immediately at the neutralization point (pH 7). The solution was concentrated to 50 ml under reduced pressure to yield fine crystals (main portion was XVIII). After filtration, the yellow filtrate was acidified with dilute HCl and the resultant precipitates were collected and recrystallized from ethanol to yield colourless needles of XVIII, m.p. 170–171° (500 mg), $[\alpha]_D^{27}$ 0° ± 1·5° (CH_3OH), λ_{max} 299 mμ (log ε 4·52), (Found: C, 67·06; H, 7·19; N, 3·63. $C_{24}H_{29}O_6N$ requires: C, 67·43; H, 6·84; N, 3·28%). Measurement of the acidity constant (pKa) by the titration method was undertaken, but no consumption of alkali by the sample was observed below pH 10·5.

Action of acetic anhydride on lactol (XVIII)

To a mixture of 1 ml of pyridine and 5 ml of acetic anhydride 50 mg of XVIII was added. It was kept at 70° for ten min and after cooling, water was added to the solution, the resultant crystals were collected, recrystallized from ethanol and identified as the starting material, XVIII. The same procedure was applied with prolonged heating (1 hr); the resultant crystals, differing from XVIII,

were recrystallized from ethanol and were identified as N-acetyl isoaureothamine by the comparison of the infra-red spectra, m.p. 193–194°, $[\alpha]_D^{28}$ 0° ± 2° ($CHCl_3$—CH_3OH (1:2)), [cf. N-acetyl isoaureothamine prepared from aureothin, m.p. 214–215°, $[\alpha]_D^{28}$ +140° ± 1·5° ($CHCl_3$—CH_3OH (1:2)]. On mixing two species of N-acetyl isoaureothamine (optical active and inactive species), no depression of m.p. was observed; mixed melting point, 193–200°, (Found: C, 70·59; H, 6·50. $C_{24}H_{27}O_5N$ requires: C, 70·40; H, 6·50%).

Racemic N-acetyl isoaureothamine was also obtained by treatment of XVIII with diazomethane.

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