METABOLIC PRODUCTS OF MICROORGANISMS. 222[1] β-OXOTRYPTAMINE DERIVATIVES ISOLATED FROM *STREPTOMYCES RAMULOSUS*

YONGLE CHEN,* AXEL ZEECK**

Institut für Organische Chemie der Universität
Tammannstr. 2, D-3400 Göttingen, F.R.G.

ZENGXIANG CHEN* and HANS ZÄHNER

Institut für Biologie II,
Lehrstuhl Mikrobiologie I der Universität
Auf der Morgenstelle 28,
D-7400 Tübingen, F.R.G.

(Received for publication March 14, 1983)

Besides acetomycin[2~4], *Streptomyces ramulosus* Tü 34 produces several other metabolites which were extracted from the culture broth with 1-butanol at pH 8. The evaporated residue of the organic layer was separated on silica gel. In the course of our chemical screening[5~7] the TLC plates were spotted by various staining reagents. Some of the colorless metabolites turned yellow and red-violet, respectively, with EHRLICH's reagent and proved to be new antibiotics of the amicetin group[8]. Two other compounds, 34-M and 34-N, extinguished UV light on silica gel F_{254} and gave violet spots in the Barrollier test and violet-brown spots with blue tetrazolium reagent. The metabolites were identified as β-oxotryptamine derivatives and are the subject of this note.

The 34-N fraction[8] was further purified on Sephadex LH-20 with chloroform and yielded pure 34-N (0.2 mg/liter culture broth) as colorless amorphous powder. By the physico-chemical data (Table 1) 34-N was recognized as *N*-acetyl-β-oxotryptamine (**1**) whose synthesis has been recently reported[9].

The 34-M fraction[8] was chromatographed on silica gel with chloroform - methanol (9: 1). The eluate containing 34-M was evaporated and the residue triturated with acetonitrile - citrate buffer solution pH 6 (7: 3). The precipitate was filtered and crystallized from methanol to give 34-M as colorless needles (3 mg/liter culture broth). 34-M and 34-N differ in the Rf values (Table 2) while their IR and UV spectra are quite similar (Table 1, Fig. 1). The molecular formula of 34-M contains one oxygen atom more than **1** established by high resolution EI mass spectroscopy (Calcd.: 232.0847, Found: *m/z* 232.0849). 34-M was identified as *N*-acetyl-α-hydroxy-β-oxotryptamine (**2**) based on the following data.

The ^{1}H NMR spectra (Table 3) show that 34-M contains a methine proton (δ 6.20, sharpened to a singlet after D_2O exchange) instead of the methylene group (δ 4.54) of **1**. The downfield shift implies an additional hetero atom attached at

Table 2. Rf values of 34-N (**1**), 34-M (**2**) and its derivatives **3** and **4**.

Solvent system	1	2	3	4
$CHCl_3$ - MeOH (9: 1)	0.26	0.22	0.57	0.50
EtOAc - MeOH (5: 1)	0.32	0.41	0.62	0.53
Toluene - acetone (3: 2)	0.06	0.08	0.28	0.41

TLC plates (silica gel, Polygram SIL G/UV_{254}, Macherey-Nagel).

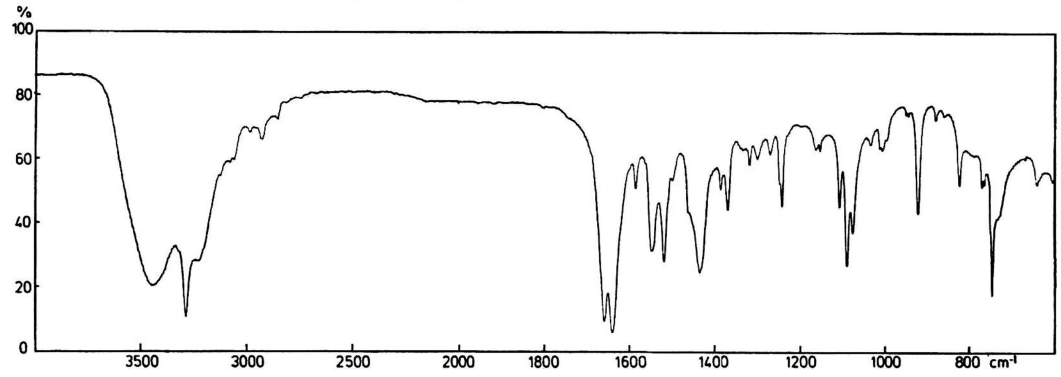
Table 1. Physico-chemical properties of 34-M (**2**) and 34-N (**1**).

	34-M (2)	34-N (1)
Melting point	146~147°C	203~204°C
$[\alpha]_D^{20}$	−92° (*c* 1.0, MeOH)	Inactive
Molecular formula	$C_{12}H_{12}N_2O_3$	$C_{12}H_{12}N_2O_2$
MW (EI-MS)	232	216
IR (KBr)	1660, 1637, 1585, 1545, 1518 cm^{-1}	1655, 1627, 1580, 1545, 1515 cm^{-1}
UV (MeOH): λ_{max} (ε)	302 (11100), 262 (8200), 242 nm (10800)	297 (10400), 259 (8200), 240 nm (11500)
EI-MS: *m/z* (abund.)	232 (<1%, $M^{+\cdot}$), 173 (8, M−C_2H_5NO), 144 (65, C_9H_6NO), 116 (20), 89 (18), 59 (80), 44 (100)	216 (14%, $M^{+\cdot}$), 144 (100), 116 (14), 89 (13)

* Present address: Sichuan Industrial Institute of Antibiotics, Shan-Ban-Qiao Road 9, Chengdu, Sichuan, VR China.

** To whom communications should be addressed.

Fig. 1. IR spectrum of 34-M (2) in KBr.



C-2′. The EI mass spectrum indicates a hydroxyl group in this position, because a McLAFFERTY rearrangement gives an ion of mass equivalent to acetamide (*m/z* 59) and a fragment peak at *m/z* 173 corresponding to 3-indoleglyoxal. The fragmentation at the carbonyl group attached to the indole nucleus afforded typical ions of the latter at *m/z* 144 and 116.

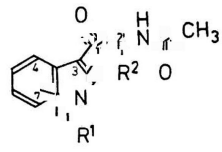
Acetylation of 34-M in acetic anhydride - pyridine (1: 2) during 4 hours at room temperature yielded the diacetate **3** which was purified on Sephadex LH-20 with chloroform. The molecular formula $C_{16}H_{16}N_2O_5$ of **3** was determined by high resolution mass spectroscopy (Calcd.: 316.1059, Found: *m/z* 316.1057). The molecular ion loses acetic acid (*m/z* 256) and ketene (*m/z* 214). In the ^{1}H NMR spectrum of **3** the methine signal is shifted downfield to δ 7.36, caused by the acetylation of the attached hydroxyl group (Table 3). The second acetyl group (δ 2.75) is bound to the indole nitrogen, deduced from the downfield shift of 2-H and 7-H. Further evidence arises from the mass spectrum which shows an additional indole peak at *m/z* 186.

Treatment of 34-M with 1 M methanolic HCl (3 hours/70°C) afforded the dimethyl acetal **4** of 3-indoleglyoxal as the main product which was purified on silica gel with chloroform - methanol (95: 5). The carbonyl group of **4** gives a strong IR absorption band at 1640 cm^{-1}. The high resolution mass spectrum proves the molecular formula $C_{12}H_{13}NO_3$ (Calcd.: 219.0895, Found: *m/z* 219.0896) and shows characteristic fragment peaks at *m/z* 144 (26%) and *m/z* 75 (100%) indicating a $CH(OCH_3)_2$ group. In the ^{1}H NMR spectrum of **4** the indole protons are comparable with the signals of 34-M (Table 3), and the acetal methine proton (δ 5.06) appears in the typical range.

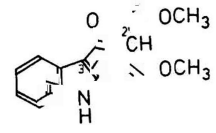
Table 3. ^{1}H NMR data[a] of 34-N (**1**), 34-M (**2**) and its derivatives **3** and **4**.

Assignment	1[b]	2[c]	3[d]	4[d,e]
2′-H_2/2′-H	4.54 s	6.20[f]	7.36 s	5.07 s
5′-H_3	2.04 s	1.91 s	2.11 s[g]	—
2-H	8.15 s	8.28 s	8.62 s	8.32 d
4-H	8.15 m	8.20 m	8.46 m	8.50 m
5-H/6-H	7.15 m	7.20 m	7.40 m	7.35 m
7-H	7.35 m	7.50 m	8.33 m	7.45 m

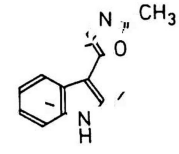
[a] δ Values in ppm relative to internal TMS.
[b] CD_3OD at 80 MHz.
[c] $(CD_3)_2SO$ at 100 MHz.
[d] $CDCl_3$ at 200 MHz.
[e] 6H-singlet for methoxyl at δ 3.53.
[f] Broad for 2H, change to 1H-singlet with D_2O; further exchangeable signals at δ 8.66 and 12.00.
[g] Further acetyl singlets at δ 2.15 and 2.75.



1 R^1=R^2=H
2 R^1=H; R^2=OH
3 R^1=Ac; R^2=OAc



4



5

The ^{13}C NMR data of 34-M (**2**) in DMSO-d_6 (50 MHz, multiplet selection) agree with the proposed structure. The upfield shift of the C-2′ signal (δ 72.7 d) is remarkable. The other signals shows no unusual deviation[7,10]: δ 190.4 s (C-1′),

169.3 s (C-4'), 136.3 s (C-7a), 134.5 d (C-2), 125.8 s (C-3a), 123.0, 122.0, 121.1 (d for C-4, C-5, C-6), 112.5 s (C-3), 112.2 d (C-7), 22.7 q (C-5').

The metabolites **1** and **2** give evidence that *S. ramulosus* produces enzymes which are able to oxidize derivatives of tryptophan or tryptamine in the side chain. A hemoprotein with this function has been isolated from Pseudomonas[11]. The existence of **1** corroborates the hypothesis that *N*-acyl-β-oxotryptamines might be bioconverted to indolyloxazoles which are more common metabolites of Streptomyces[7]. **1** conceivably is a direct precursor of pimprinine (**5**)[12] which we did not find in the extracts of our strain. The hydroxylation of **1** at C-2' is a stereospecific bioconversion, although the configuration of the chiral center is yet unknown. **2** is on the oxidation level of 3-indoleglyoxal, a known oxidation product of tryptophan after incubation with Pseudomonas side chain oxidase[13]. **1** and **2** did not exhibit antibacterial or antifungal activity.

Acknowledgments

We thank the Deutsche Forschungsgemeinschaft and the Friedrich Ebert-Stiftung for financial support.

References

1) Drautz, H.; W. Keller-Schierlein & D. Wuthier: Metabolic products of microorganisms. 221. (3*S*,8*E*)-5-Oxo-8-decen-1,3-diol, ein Stoffwechselprodukt von *Streptomyces fimbriatus*. Helv. Chim. Acta 66: in press, 1983
2) Ettlinger, L.; E. Gäumann, R. Hütter, W. Keller-Schierlein, F. Kradolfer, L. Neipp, V. Prelog & H. Zähner: Über die Isolierung und Charakterisierung von Acetomycin. Helv. Chim. Acta 41: 216~219, 1958
3) Keller-Schierlein, W.; M. Lj. Mihailovic & V. Prelog: Über die Konstitution von Acetomycin. Helv. Chim. Acta 41: 220~228, 1958
4) Bachmann, E.; H. Gerlach, V. Prelog & H. Zähner: Zur Biogenese des Acetomycins. Helv. Chim. Acta 46: 605~611, 1963
5) König, W. A.; H. Drautz & H. Zähner: Ketalin, ein Metabolit aus *Streptomyces* Tü 1668. Liebigs Ann. Chem. 1980: 1384~1391, 1980
6) Drautz, H.; H. Zähner, E. Kupfer & W. Keller-Schierlein: Isolierung und Struktur von Streptazolin. Helv. Chim. Acta 64: 1752~1765, 1981
7) Noltemeyer, M.; G. M. Sheldrick, H. U. Hoppe & A. Zeeck: 2-Ethyl-5-(3-indolyl)oxazol from *Streptomyces cinnamomeus* discovered by chemical screening. Characterization and structure elucidation by X-ray analysis. J. Antibiotics 35: 549~555, 1982
8) Chen, Y.; A. Zeeck, Z. Chen & H. Zähner: Isolation and structure of oxyplicacetin and 34-D, new members of the amicetin group. To be published.
9) Yoshioka, T.; K. Mohri, Y. Oikawa & O. Yonemitsu: Synthesis of oxazolylindole alkaloids from tryptamine and tryptophan by oxidation with 2,3-dichloro-5,6-dicyanobenzoquinone. J. Chem. Res. (S) 1981: 194~195, 1981
10) Shemma, M. & D. M. Hindenlang: Carbon-13 NMR Shift Assignments of Amines and Alkaloids. Plenum Press, New York, 1979
11) Takai, K.; H. Ushiro, Y. Noda, S. Narumiya, T. Tokuyama & O. Hayaishi: Crystalline hemoprotein from *Pseudomonas* that catalyzes oxidation of side chain of tryptophan and other indole derivatives. J. Biol. Chem. 252: 2648~2656, 1977
12) Joshi, B.; W.I. Taylor, D. S. Bhate & S. S. Karmarkar: The structure and synthesis of pimprinine. Tetrahedron 19: 1437~1439, 1963
13) Narumiya, S.; K. Takai, T. Tokuyama, Y. Noda, H. Ushiro & O. Hayaishi: A new metabolic pathway of tryptophan initiated by tryptophan side chain oxidase. J. Biol. Chem. 254: 7007~7015, 1979