2-ETHYL-5-(3-INDOLYL)OXAZOLE FROM *STREPTOMYCES CINNAMOMEUS* DISCOVERED BY CHEMICAL SCREENING

CHARACTERIZATION AND STRUCTURE ELUCIDATION BY X-RAY ANALYSIS

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In the lipophilic extracts from *Streptomyces cinnamomeus* 2-ethyl-5-(3-indolyl)oxazole (**1a**) was detected by chemical screening methods. The structure of the crystalline **1a** was determined by spectroscopic and X-ray analysis. The new mono- and dibromo derivatives **1b** and **1c** are described. **1a** is identical with pimprinethine and belongs to a group of microbial indole alkaloids, which can be regarded as masked tryptamine derivatives.

An example of the diversity of *Streptomyces* metabolism is the observation that individual strains produce different secondary metabolites at the same time. If these metabolites differ fundamentally in structural type and activity spectrum, then there is a risk even with known strains of overlooking other types of natural products by one-sided screening.

Secondary metabolites produced under specified culture conditions in appropriate quantity can only be recognized with relative certainty when the thin-layer chromatograms of the various isolated raw products are stained with appropriate reagent sprays. Special staining reagents offer the possibility of identifying chosen classes of compounds[1~6]. By a combination of several reagents which show a broad response to common substance classes, one may obtain an overall picture of the pattern of products from a single strain. In the chemical screening procedures the biological activity of the secondary metabolites is not immediately taken into account.

The antibiotic kirrothricin[7,8] constitutes the major component of the lipophilic raw product extracted from the culture filtrate of *Streptomyces cinnamomeus* (strain Tü 89). As byproducts were identified aureothin[9] and a weak base, which extinguished in UV-light on silica gel F_{254}, turned blue-green in the Barrollier-test[10] and red-violet with EHRLICH's reagent. We describe below the characteristics and the bromination of this compound, together with a crystal structure analysis, which identified it as 2-ethyl-5-(3-indolyl)oxazole (**1a**).

Physico-chemical Characterization

1a was separated by preparative layer chromatography on silica gel with chloroform - methanol (9: 1) out of the leading zone obtained on isolation of kirrothricin, and purified by recrystallization from

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Fig. 1. IR spectrum of **1a** in KBr.

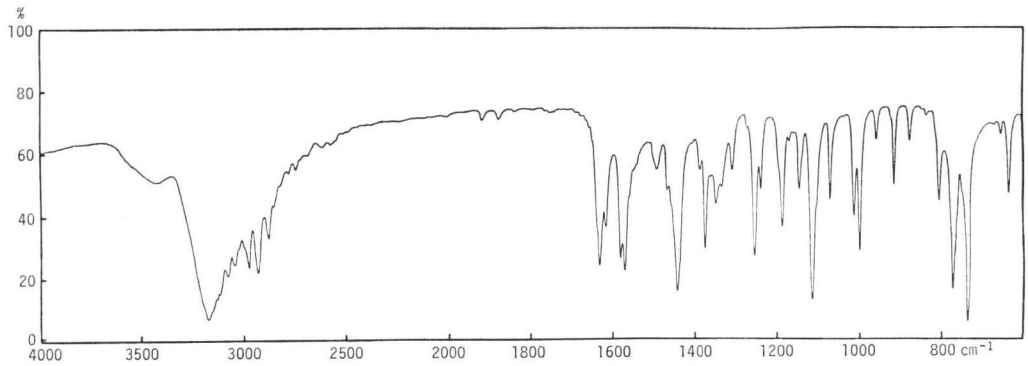
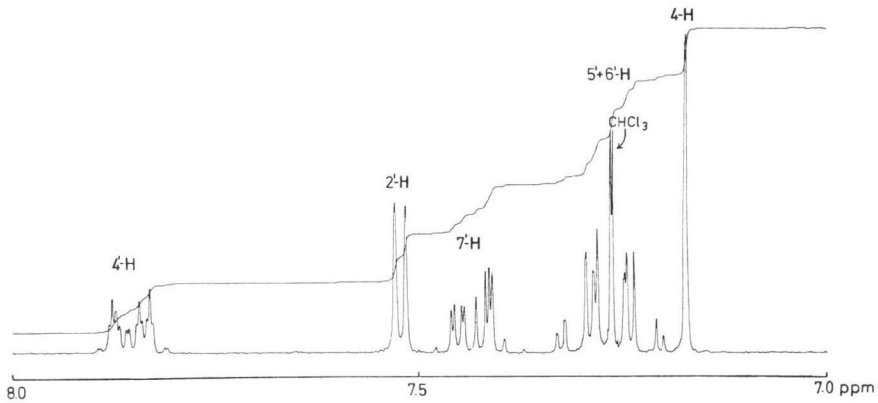


Fig. 2. PMR spectrum of **1a** in $CDCl_3$ (200 MHz, region of aromatic protons).



chloroform. The elementary analysis and mass spectrum prove the formula $C_{13}H_{12}N_2O$ (M. W. 212) for the colorless, optically inactive **1a**. The IR spectrum (Fig. 1) shows no intense absorption in the carbonyl region. The UV spectrum is identical in neutral and basic environments. The band at long wavelength exhibits a bathochromic shift with modified extinction coefficient in acids.

The PMR spectrum (Table 1) shows, in addition to an ethyl group, resonances for six aromatic H and one NH. The δ values and assumed coupling constants of the aromatic protons, when input to a LAOCOON simulation (LAME, Varian), yielded a pattern which agreed very well with the observed spectrum (Fig. 2). Noteworthy is the deshielding ($\Delta\delta=0.18\sim0.32$ ppm) compared with indole of all protons

Table 1. PMR data of 2-ethyl-5-(3-indolyl)oxazole (**1a**) in $CDCl_3$ (δ values in ppm).

Position	PMR (200 MHz)	CMR (25.2 MHz)
2	—	164.6 s
4	7.18 s	123.4 d[b]
5	—	149.5 s
6	2.90 q (J=7.5 Hz)	22.2 t
7	1.43 t (J=7.5 Hz)	11.5 q
1'-NH	8.83 (broad)	—
2'	7.52 d (J=2.6 Hz)	123.1 d
3'	—	105.0 s
3'A	—	125.0 s
4'	7.85 m[a]	120.1 d
5'	7.25 m	118.1 d
6'	7.29 m	121.0 d
7'	7.44 m	112.5 d
7'A	—	137.8 s

a) Coupling constants in Hz derived from the LAOCOON simulation: 4'/5'=7.8; 4'/6'=1.3; 4'/7'=0.9; 5'/6'=7.1; 5'/7'=0.9; 6'/7'=8.1; 4'/1'-NH=0.8.

b) The assignment of all doublets is not quite certain.

except 7′-H. Double resonance experiments on **1a** show an intramolecular nuclear Overhauser effect (NOE) of nearly 20% between 4-H and 4′-H, while there is no NOE enhancement to be seen for 2′-H. By correlation of this NOE with the internuclear distance of the protons 4-H/4′-H we calculate[11] 2.53 Å, whereas the same distance derived from X-ray data is 2.28 Å (assuming C-H=1.08 Å).

We conclude that the *S-cis* conformation shown in formula **1a** and found in the crystal is also adopted in solution, but with appreciable torsional motion about C(3′)-C(5). The CMR spectrum (Table 1) is consistent with the structure **1a**. The resonances are assigned in comparison with tryptamine[12].

Bromination of **1a** with elementary bromine in chloroform in the presence of iron, or with 2,4,4,6-tetrabromocyclohexa-2,5-dienone[13] in chloroform, yielded the bromine derivatives **1b** and **1c**, for which the structures could be derived primarily from the PMR data. As would be expected, the oxazole ring is brominated before the indole ring.

X-Ray Analysis

Single crystals of 2-ethyl-5-(3-indolyl)oxazole (**1a**) in the form of colorless plates were obtained by slow cooling of warm saturated chloroform solutions. The crystals are monoclinic $P2_1/n$, $Z=4$, $a=11.592$ (15), $b=7.098$ (10), $c=13.257$ (18) Å, $\beta=90.58$ (10)°, U=1090.7 Å^3. The structure was solved by multisolution tangent refinement. The assignment of the nitrogen and oxygen atoms, and the location of the hydrogen atoms, proved possible from the X-ray data alone. Fig. 3 shows a difference electron density synthesis calculated in the least-square plane passing through the non-hydrogen atoms. For the purposes of the preceding refinement in which the high-angle data were given increased weight, O(1) and N(3) were given nitrogen scattering factors and N(1′) was input as carbon. The electron density map clearly shows all the hydrogen atoms which lie in the plane, together with excess electron density

Fig. 3. Difference electron density in the molecular plane based on a high-angle refinement of the heavy atoms.

All atoms were considered to be carbon in this refinement except for N(3) and O(1) which were given nitrogen scattering-factors. The hydrogen atoms and excess electron density at N(1′) and O(1) (indicating that their scattering-factors should be increased by one electron) can be seen clearly.

Fig. 4. The molecule of **1a**, showing bond lengths (with standard deviations) in Å.

Bond angles in degrees are: O1–C2–N3 113.2(2), O1–C2–C6 116.5 (2), C2–N3–C4 105.0 (2), N3–C4–C5 109.5 (2), N3–C2–C6 130.2 (2), C2–C6–C7 112.5 (2), O1–C5–C4 106.7 (2), O1–C5–C3′ 116.7 (2), C4–C5–C3′ 136.5 (2), C5–C3′–C2′ 125.3 (2), C5–C3′–C3′A 127.8 (2), C2′–C3′–C3′A 106.9 (2), N1′–C2′–C3′ 109.8 (2), C2′–N1′–C7′A 109.3 (2), N1′–C7′A–C7′ 130.3 (2), N1′–C7′A–C3′A 107.8 (2), C3′–C3′A–C7′A 106.1 (2), C3′–C3′A–C4′ 135.2 (2), C3′A–C4′–C5′ 119.1 (2), C4′–C5′–C6′ 121.3 (3), C5′–C6′–C7′ 121.2 (2), C6′–C7′–C7′A 117.9 (2), C7′–C7′A–C3′A 121.8 (2), C2–O1–C5 105.5 (2), and C7′A–C3′A–C4′ 118.7 (2).

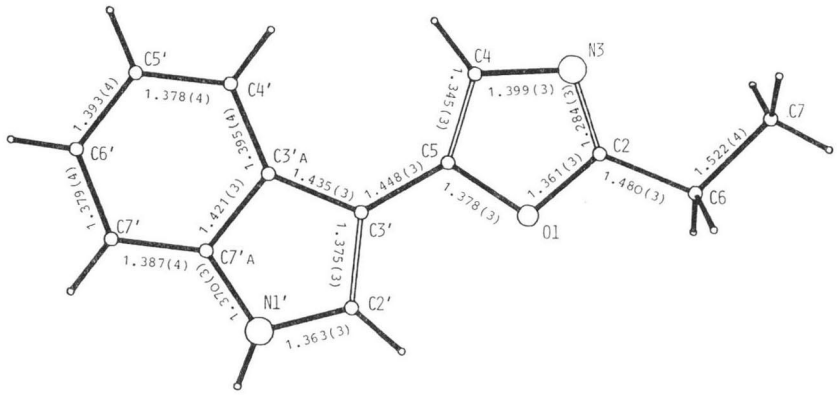
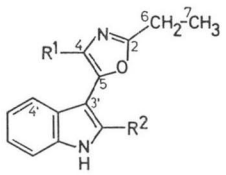
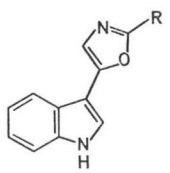


Table 2. Atomic coordinates (estimated standard deviations in parentheses) for nonhydrogen atoms.

Atom	x	y	z
O(1)	0.0052 (1)	0.2327 (3)	0.5717 (1)
C(2)	0.0740 (2)	0.2267 (4)	0.6555 (1)
N(3)	0.1808 (2)	0.2564 (4)	0.6366 (1)
C(4)	0.1849 (2)	0.2856 (4)	0.5323 (2)
C(5)	0.0779 (2)	0.2715 (4)	0.4928 (1)
C(6)	0.0162 (2)	0.1873 (4)	0.7523 (2)
C(7)	0.1001 (3)	0.1852 (5)	0.8413 (2)
N(1′)	−0.1078 (2)	0.2707 (4)	0.2702 (1)
C(2′)	−0.0888 (2)	0.2518 (5)	0.3713 (1)
C(3′)	0.0256 (2)	0.2841 (3)	0.3934 (1)
C(3′A)	0.0812 (2)	0.3258 (3)	0.2998 (2)
C(4′)	0.1931 (2)	0.3723 (4)	0.2714 (2)
C(5′)	0.2161 (2)	0.4020 (5)	0.1709 (2)
C(6′)	0.1298 (2)	0.3872 (4)	0.0975 (2)
C(7′)	0.0180 (2)	0.3426 (4)	0.1228 (2)
C(7′A)	−0.0059 (2)	0.3135 (4)	0.2239 (2)





1a $R^1=R^2=H$ **2a** $R=CH_3$
1b $R^1=Br$; $R^2=H$ **2b** $R=CH_2-C_6H_5$
1c $R^1=R^2=Br$

at N(1′) and O(1), enabling these atoms to be assigned to nitrogen and oxygen respectively. The assignment was confirmed by the observed bond lengths. Refinement with individual isotropic temperature factors, a rigid methyl group, a riding model (C-H=0.96 Å) for the remaining hydrogen atoms, and the other atoms anisotropic, converged to $R=0.064$ for 1730 unique data with $F>4\sigma(F)$. Final atomic coordinates are presented in Table 2; Fig. 4 shows a view of the molecule.

Discussion

1a is identical to pimprinethine, which was isolated recently from *Streptoverticillium olivoreticuli*[14] and structurally elucidated by synthesis[14,15]. **1a** is related to pimprinine (**2a**), which is produced by *Streptomyces pimprina*[16]. Pimprinine shows antiepileptic[17] and monoamine oxidase inhibitory activities[18], both of which we expect for **1a** too. The 5-(3-indolyl)oxazoles can be looked upon as masked

tryptamines. The X-ray analysis and PMR data indicate that the *S-cis* conformation of the coplanar ring systems is favoured. The distance of N(3) to N(1′) in the conformer **1a** is 5.872 (4) Å and is certainly important for the pharmacological effect. An antibacterial or antifungal activity for **1a** and its bromine derivatives could not be proved.

1a, **2a** and pimprineaphine (**2b**)[14] are microbial indole alkaloids and apparently more common than assumed until now. The compounds illustrate that it is difficult to detect all interesting secondary metabolites of a *Streptomyces* strain with the help of biological screening methods alone. Chemical screening methods are simpler, and after isolation and structure elucidation new metabolites can be subject to selected biological tests.

Experimental

General

IR spectra in pressed KBr disks were recorded using a Perkin Elmer model 297 spectrometer, UV spectra using a Zeiss DMR 21 spectrometer. PMR spectra were determined at 200 MHz with a Varian XL-200, the FT-CMR spectrum at 25.2 MHz on a Varian XL-100. Chemical shifts (δ in ppm) are reported relative to internal TMS. EI-mass spectra were obtained on a Varian MAT 731 instrument (70 eV) using the direct probe insert, high resolution with perfluorokerosine as a standard. All molecular formulae were in agreement with the high resolution data. Melting points were established on a heated microscope (Reichert, Austria) and were corrected. Thin-layer chromatography (TLC) was performed on silica gel plates (Merck 60 F_{254}), preparative TLC on silica gel (Macherey & Nagel, P UV 254, plates 20×40 cm, layer 2 mm). The X-ray analysis was carried out with MoKα radiation on a Stoe two-circle diffractometer (layers *h* 0–7 *l*). 2916 unique reflexions were measured. All calculations were performed with programs written by G.M.S. for the Data General Eclipse mini-computer. Tables of thermal parameters, hydrogen atom coordinates and observed and calculated structure factors are available on request from the authors.

Isolation of 2-Ethyl-5-(3-indolyl)oxazole (**1a**)

Purification of 3.1 g crude kirrothricin[7] yielded 912 mg of a mixture of **1a** and aureothin in the faster running fraction. Further purification was achieved by preparative TLC on silica gel with chloroform - methanol (9: 1) as eluant. Under UV light (254 nm) two separate zones could be seen. From the slower running zone 356 mg (14 mg/liter culture broth) **1a** could be eluted. Crystallization was achieved by cooling down a solution of **1a** in boiling chloroform over 2 days, yielding colorless crystals, mp 161°C. Rf 0.36 (TLC, ether). **1a** is soluble in methanol, slightly soluble in chloroform or acetone, insoluble in pentane or water. IR (Fig. 1): 1633, 1617, 1582, 1572 cm^{-1}. UV (MeOH): λ_{max} (ε) 295 sh, 278 sh, 266 (14100), 224 nm (22200). UV (MeOH - HCl): λ_{max} (ε) 304 (19900), 283 sh, 270 sh, 219 nm (23800). PMR and CMR see Table 1. Ms: *m/z* (abund.)=212 (100%, $M^{+\cdot}$, $C_{13}H_{12}N_2O$), 197 (36%, $M-CH_3$), 183 (10%, $M-C_2H_5$), 170 (6%), 169 (13%), 157 (22%), 156 (24%), 142 (38%), 130 (18%), 89 (13%).

Anal. Calcd. for $C_{13}H_{12}N_2O$: C 73.57, H 5.70, N 13.2%.
Found: C 73.34, H 5.47, N 13.0%.

4-Bromo-2-ethyl-5-(3-indolyl)oxazole (**1b**)

a) A solution of 200 mg (0.47 mmole) 2,4,4,6-tetrabromocyclohexa-2,5-dienone in 10 ml chloroform was added to a solution of 100 mg (0.47 mmole) **1a** in 10 ml chloroform. After 2 hours at −10°C reaction was completed. After addition of 10 ml water the pH was adjusted to 9 with 2 N NaOH and the mixture was extracted with chloroform three times. The organic layer was dried over with sodium sulfate (anhydrous) and evaporated. The residue was purified by chromatography on silica gel with ether as current system. From the main zone (visible under UV light at 254 nm) **1b** was eluted and precipitated from chloroform with *n*-pentane yielding 90 mg (52%) as colorless crystals, mp 158°C. Rf 0.63 (TLC, ether). IR: 1626, 1607, 1566 cm^{-1}. UV (MeOH): λ_{max} (ε) 322 sh, 300 sh, 288 (13900), 272 (14600), 222 nm (31200). PMR ($CDCl_3$): δ 1.42/2.92 (t/q, J=7.5 Hz, 2-CH_2CH_3), 7.27 (m, 5′-H/6′-H), 7.45 (m, 7′-H), 7.88 (d, J=2.7 Hz, 2′-H), 8.07 (m, 4′-H), 8.47 (broad, NH). MS: *m/z* (abund.)= 292/290 (98%, $M^{+\cdot}$), 277/275 (10%, $M-CH_3$), 263/261 (2%, M−29), 235/233 (7%), 183 (100%), 168

(8%), 156 (18%), 144/142 (34%), 128 (13%), 116 (20%). 89 (20%), 56 (27%).

Anal. Calcd. for $C_{13}H_{11}N_2OBr$: C 53.63, H 3.81, N 9.6, Br 27.4%.
Found: C 53.84, H 3.84, N 9.7, Br 27.5%.

b) A solution of 24 μl (0.94 mmole) bromine in 5 ml chloroform was added dropwise to a solution of 100 mg (0.47 mmole) **1a** in 10 ml chloroform containing 50 mg iron dust. The mixture was stirred at room temperature for 2 hours. After washing with water and evaporation of the dried organic layer a yellowish residue was obtained which was purified by preparative TLC on silica gel with ether as eluant. There are seen three main zones under UV light (254 nm), the slowest one contained unreacted **1a** (15 mg). The middle zone yielded 80 mg (69%) **1b** which was identical with the compound described above.

4,2'-Dibromo-2-ethyl-5-(3-indolyl)oxazole (**1c**)

The quickest running zone from the forgoing experiment (b) yielded 20 mg (14%) **1c**, which crystallized upon standing, mp 183°C. Rf 0.71 (TLC, ether). IR: 1640, 1617w, 1580w, 1560 cm^{-1}. UV (MeOH): λ_{max} (ε) 288 sh, 280 (10300), 272 sh, 218 nm (27000). UV (MeOH - HCl): λ_{max} (ε) 288 (9500), 280 (10300), 272 (10100), 218 nm (26600). UV (MeOH - NaOH): λ_{max} (ε) 305 sh, 288 (9500), 279 (9600), 272 sh, 230 sh nm. PMR ($CDCl_3$): δ 1.40/2.89 (t/q, J=7.5 Hz, 2-CH_2CH_3), 7.23 (m, 5'-H/6'-H), 7.37 (m, 7'-H), 7.57 (m, 4'-H), 8.50 (s, broad, NH). MS: m/z (abund.)=372/370/368 (100%, $M^{+\cdot}$, $C_{13}H_{10}N_2OBr_2$), 315/313/311 (6%), 263/261 (60%), 235/233 (11%), 224/222/220 (12%), 208/206 (5%), 182 (28%).

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