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STRUCTURE DETERMINATION OF INDOLOCARBAZOLE ALKALOIDS BY NMR SPECTROSCOPY

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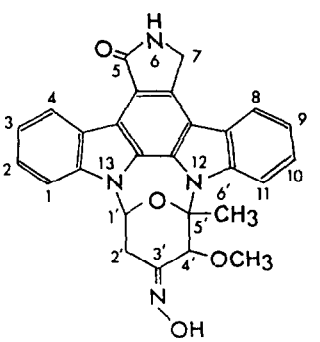
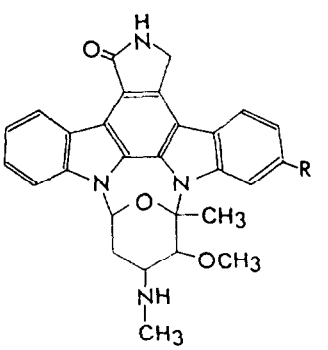
Abstract: The structures of two indolocarbazole alkaloids with macrophage-activating properties, TAN-1030 A and TAN-999, were determined based on NMR spectral analysis.

TAN-1030 A and TAN-999 are new indolocarbazole alkaloids produced by *Streptomyces* sp. C-71799 and *Nocardiopsis dassonvillei* C-71425, respectively[1]. Compounds having the same chromophore have been reported such as staurosporine[2,3], which was also produced by *Streptomyces* sp. C-71799, SF-2370[4], K-252 a,b,c and d[5], UCN-01[6] and so on. TAN-1030 A and TAN-999 are the first reported compounds in this series having macrophage-activating properties[1]. This paper deals in detail with the structure determination of TAN-1030 A and TAN-999 (Fig.1) using NMR techniques.

Fig. 1. Structures of TAN-1030 A, TAN-999 and staurosporine.



TAN-1030 A



TAN-999 R=OCH_3

Staurosporine R=H

Structure determination of TAN-1030 A

TAN-1030 A was obtained as colorless crystals with a melting point of 290-295°C (dec). The molecular formula of TAN-1030 A was determined to be $C_{27}H_{22}N_4O_4$ on the basis of secondary ion mass spectrum [m/z 467; $(M+H)^+$],

Table 1. ^{1}H NMR spectral data of TAN-999 and TAN-1030A (300 MHz)[a].

Position	TAN-999[b]	TAN-1030A[c]	Staurosporine[c]
1	7.27 (d,J=8.0)	7.70 (d,J=8.2)	7.56 (d,J=8.1)
2	7.46 (t)	7.50 (t)	7.45 (t)
3	7.35 (t)	7.32[d] (t)	7.27[d] (t)
4	9.40 (d,J=7.8)	9.31 (d,J=7.8)	9.30 (d,J=7.9)
6	6.28[e] (s)	8.58 (s)	8.53 (s)
7	4.96 (ABq,J=17.0)	4.96 (s like)	4.96 (s like)
8	7.75 (d,J=8.5)	7.98 (d,J=8.0)	7.96 (d,J=7.3)
9	6.96 (dd,J=2.0,8.5)	7.32[d](t)	7.27[d](t)
10		7.44 (t)	7.41 (t)
11	7.46 (d,J=2.0)	8.01 (d,J=9.1)	7.98 (d,J=8.4)
1'	6.53 (d,J=5.6)	7.04 (d,J=5.2)	6.68 (s like)
2'	2.38 (m)	3.01 (dd,J=5.2,14.0)	2.49 (m)
	2.75 (dd,J=4.0,14.7)	3.63 (d,J=14.0)	
3'	3.35 (m)		3.24 (m)
4'	3.87 (d,J=3.5)	4.73 (s)	4.04 (d,J=3.2)
6'	2.33 (s)	2.47 (s)	2.29 (s)
3'-NCH_3	1.57 (s)		1.44 (s)
4'-OCH_3	3.44 (s)	3.43 (s)	3.32 (s)
10-OCH_3	3.95 (s)		
3'=NOH		10.45 (s)	

a: Coupling constants in Hz are given in parentheses. b: In $CDCl_3$.
c: In DMSO-d_6. d: These signals overlapped.
e: This signal shifted according to the concentration.

Table 2. ^{13}C NMR spectral data of TAN-999 and TAN-1030 A (75 Mz).

Position	TAN-999[a]	TAN-1030A[b]	Position	TAN-999[a]	TAN-1030A[b]
1	106.89 d	108.88 d	11	100.72 d	115.58 d
2	124.88 d	125.21 d	11a	141.04 s	139.82 s
3	119.65 d	119.52 d	12a	130.63 s	128.03 s
4	126.50 d	125.61 d	12b	127.09 s	124.60 s
4a	123.55 s	122.85 s	13a	136.67 s	136.01 s
4b	114.88 s	114.98 s	1'	80.12 d	82.17 d
4c	118.58 s	119.15 s	2'	30.29 t	29.71 t
5	173.81 s	171.77 s	3'	50.36 d	145.12 s
7	45.92 t	45.30 t	4'	84.26 d	83.57 d
7a	131.48 s	132.25 s	5'	91.06 s	96.16 s
7b	114.24 s	114.02 s	6'	29.69 q	28.59 q
7c	118.92 s	123.79 s	3'-NCH_3	33.38 q	
8	120.94 d	120.72 d	4'-OCH_3	57.24 q	58.29 q
9	107.94 d	120.13 d	10-OCH_3	55.78 q	
10	157.49 s	124.64 d			

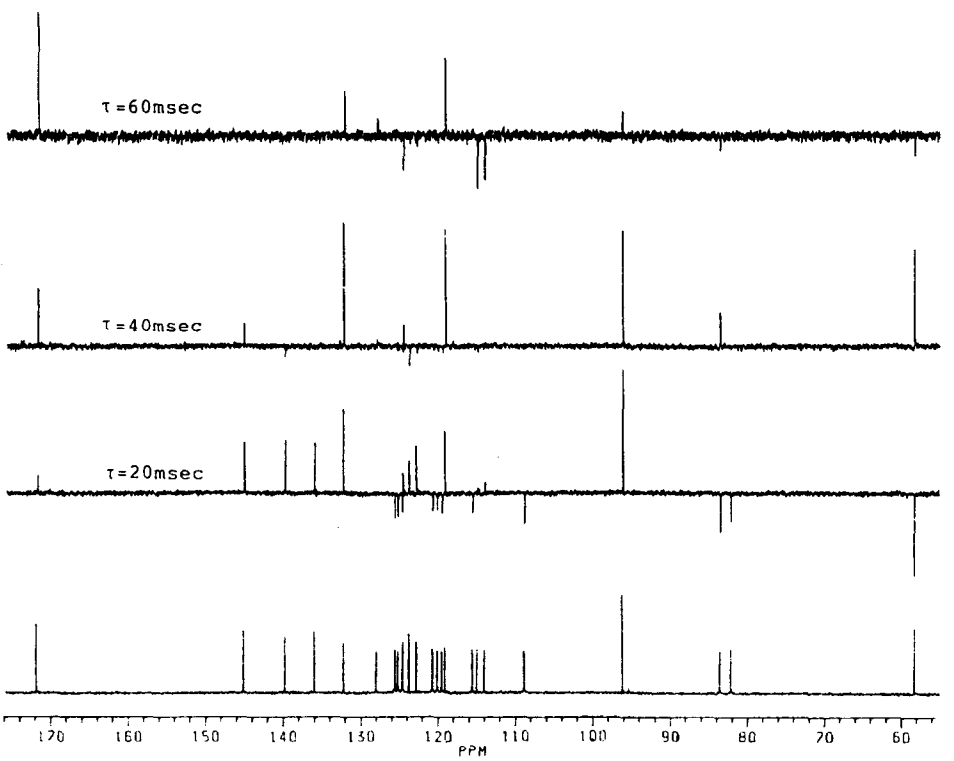
a: In $CDCl_3$. b: In DMSO-d_6.

elemental analysis (Calcd for mono-hydrate: C,66.93;H,4.99;N,11.56, Found: C,67.23; H,5.07;N,11.70) and ^{13}C NMR spectrum. The IR spectrum showed the presence of NH and OH (3430cm^{-1}) and amide (1680cm^{-1}) functions. UV absorption maxima in methanol were observed at 233nm (ε 29,400), 244

(sh,28,000), 263 (sh,31,300), 275 (sh,42,000), 289 (71,000), 319 (sh,13,400), 333 (17,700), 352 (12,100) and 369 (13,400) showing that TAN-1030 A has a chromophore similar to the indolocarbazole moiety in staurosporine[2]. TAN-1030 A showed positive color reactions with Barton and Ehrlich reagents and negative color reactions with ninhydrin and Dragendorff reagents.

^{1}H and ^{13}C NMR data are summarized in Tables 1 and 2. The data indicate the presence of one amide group, nineteen sp^2 carbons (eleven of the nineteen are quaternary carbons), one quaternary carbon, two methines, two methylenes, one methyl and one methoxy group. TAN-1030 A contains many quaternary carbons and heteroatoms. Therefore from ^{1}H-^{1}H correlation spectroscopy (COSY) only four partial structures were revealed: Ia, $-CONHCH_2-$ (H-6,8.58 and H-7,4.96); IIa,-CH=CH-CH=CH- (H-1,7.70; H-2,7.50; H-3,7.32 and H-4,9.31); IIIa,-CH=CH-CH=CH- (H-8,7.98; H-9,7.32; H-10,7.44 and H-11,8.01) and IVa,$-\overset{1}{C}HCH_2-$ (H-1',7.04 and H-2',3.01, 3.63). The assignment of proton-bonded carbon signals was achieved by ^{1}H-^{13}C COSY. To connect with other moieties which were cut off by quaternary carbons or heteroatoms, a correlation spectroscopy via long range couplings[7] (COLOC)

Fig. 2. INEPT spectra of TAN-1030 A.



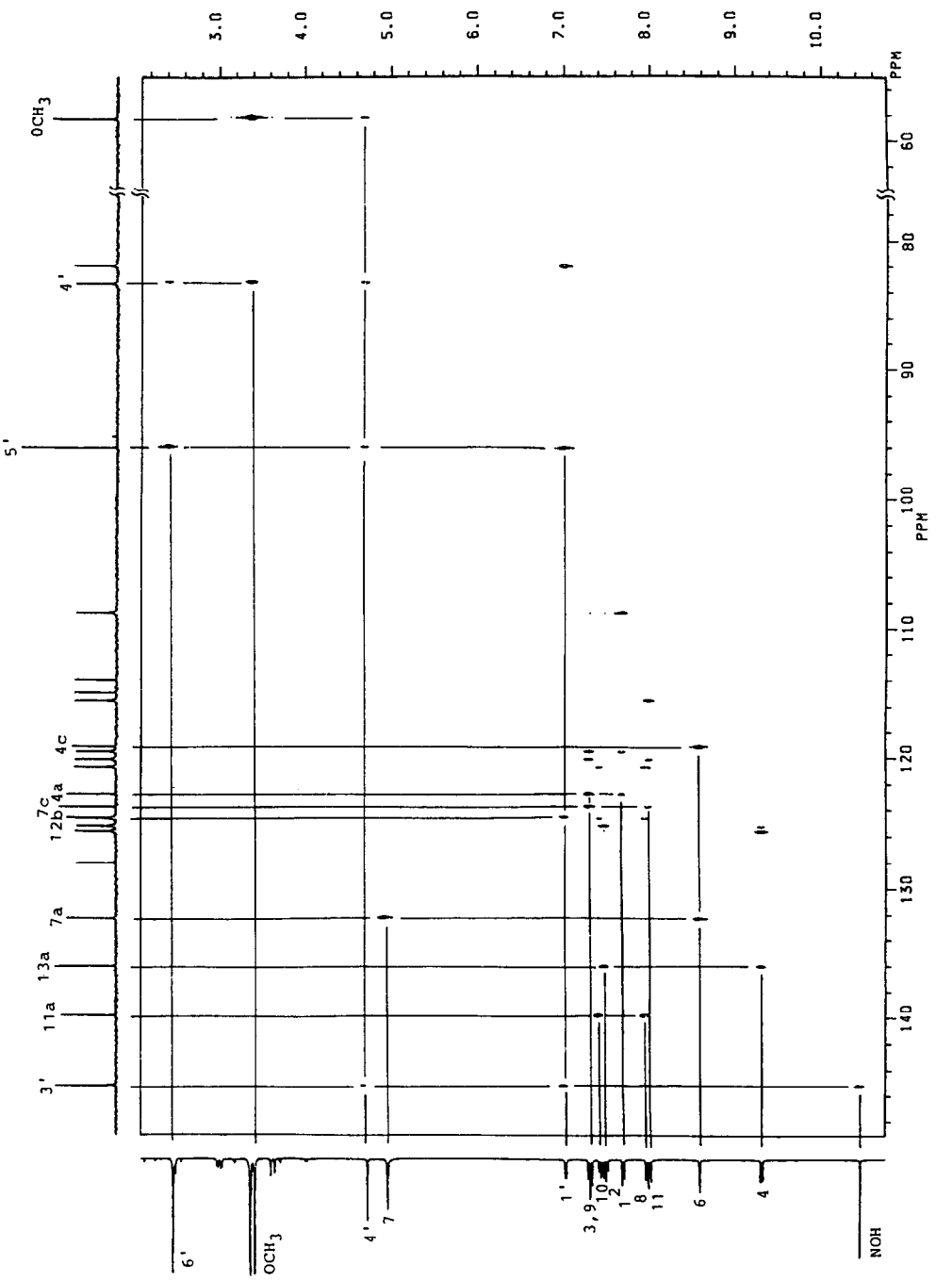
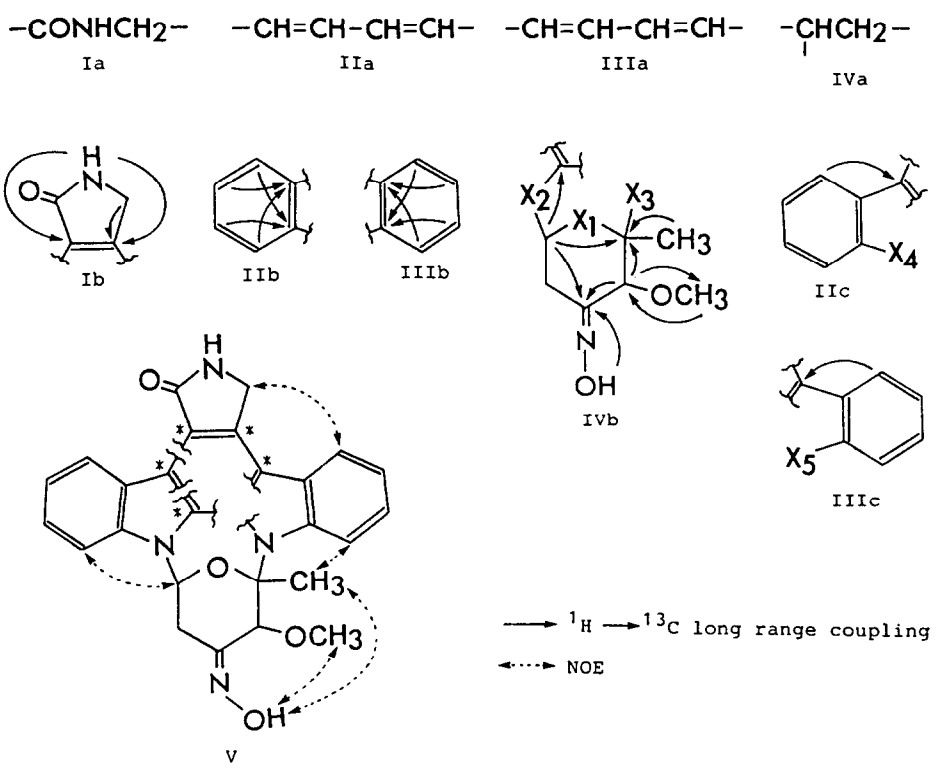


Fig. 3. COLOC spectrum of TAN-1030 A (J=12.5Hz).

was used. For a COLOC experiment, the choice of the C-H long range coupling is a difficult point. This optimization was performed via a refocussed INEPT[8] (intensive nuclei enhanced by polarization transfer) experiment (Fig.2). In this experiment the enhancement of carbon signals is best observed when the delay τ is set equal to $1/4J_{CH}$. We varied the delay τ (20, 40 and 60msec) to find the spectrum that gave the best intensity particulary for quaternary carbon signals. We chose 20msec as the delay τ giving the most quaternary carbon signals. Therefore, the C-H long range coupling was set equal to 12.5 Hz in the COLOC experiment (Fig.3).

The result of the COLOC experiment expanded the partial structures as follows (Fig.4). Two quaternary carbons (C-4c,119.15 and C-7a,132.25) coupled with a methylene (H-7,4.96) and an amide proton (H-6,8.58) in Ia. These data extended the structure Ia to a γ-lactam (Ib). Two quaternary carbons (C-4a,122.85 and C-13a,136.01) were correlated with IIa, which indicated a 1,2-disubstisuted benzene (IIb). IIIa also formed a 1,2-disubstituted benzene (IIIb) with two quaternary carbons (C-7c,123.79 and C-11a,139.82). A methoxy carbon (δ58.29) coupled with a methine (H-4',4.73)

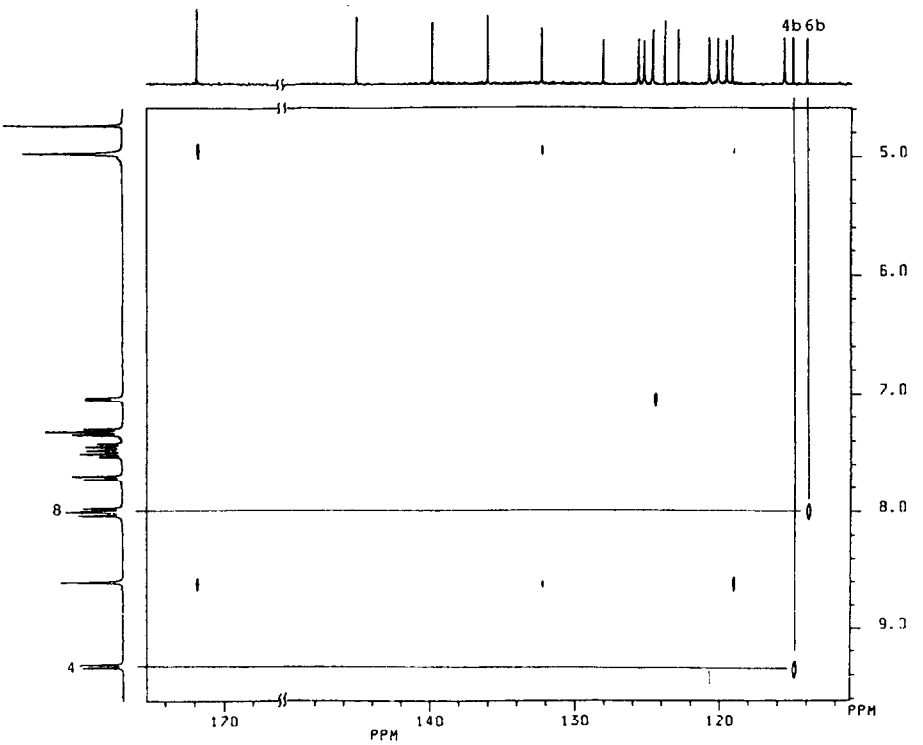
Fig. 4. Partial structures of TAN-1030 A.



and the methine carbon (C-4',83.57) coupled with the methoxy protons (δ3.43). This indicates a methoxymethine moiety. Long range coupling was observed between a quaternary carbon (C-3',145.12) and a deuterium oxide exchangeable proton (δ10.45), which revealed an oxime moiety. The oxime carbon was also correlated with the methine (H-4') of the methoxymethine moiety and a methine (H-1',7.04) of IVa. This indicates that IVa and the methoxymethine moiety are connected through the oxime group. Furthermore a quaternary carbon (C-5',96.16) coupled with a methyl group (H-6', 2.47), the methine (H-4') of the methoxymethine and the methine (H-1') of IVa. These data showed that IVa, the methoxymethine and the methyl group were combined via the quaternary carbon (C-5'). In addition, a quaternary carbon (C-12b,124.60) was correlated with the methine (H-1') of IVa. All of these data extended IVa to a six-membered ring moiety (IVb). Downfield shifts of the methine (C-1',82.17) and the quaternary carbon (C-5',96.16) in IVb indicate that these carbons are bound to heteroatoms (X_1, X_2 and X_3).

From the COLOC experiment (J=12.5Hz) ten of the thirteen quaternary carbons were connected. Two of remaining three quaternary carbons

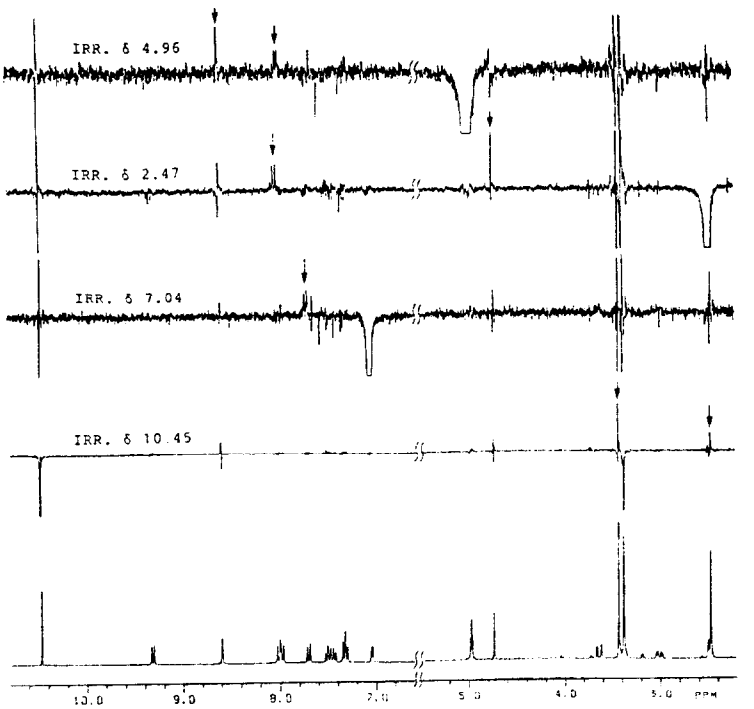
Fig. 5. COLOC spectrum of TAN-1030 A (J=4.2Hz).



(δ114.98, 114.02 and 128.03) were observed in the refocussed INEPT experiment when the delay τ was set equal at 60msec (Fig.2). Thus in the next COLOC experiment the C-H long range coupling was set at 4.2Hz (Fig.5). As expected, in this COLOC spectrum two quaternary carbons (C-4b,114.98 and C-6b,114.02) coupled with aromatic protons in IIb (H-4,9.31) and IIIb (H-8,7.98), respectively. Therefore IIb and IIIb were extended to IIc and IIIc. Downfield shifts of two quaternary carbons in IIc (C-13a,136.01) and IIIc (C-11a,139.82) show that these carbons are bound to heteroatoms (X_4 and X_5).

To connect the partial structures (Ib, IIc, IIIc and IVb), nuclear Overhauser effect (NOE) differential experiments followed (Fig.6). When the methylene (H-7,4.96) of Ib was irradiated, the aromatic proton (H-8,7.98) of IIIc and the amide proton (H-6,8.58) were enhanced. In addition the irradiation of the methyl (H-6',2.47) and the methine (H-1',7.04) enhanced the aromatic protons in IIIc (H-11,8.01) and IIc (H-1,7.70), respectively. These data showed that Ib, IIIc, IVb and IIc were connected in this order. Thus X_2 is equal to X_4 and X_3 is equal to X_5. Furthermore, when the oxime proton (δ 10.45) was irradiated, NOE enhancement was observed at the methoxy (δ 3.43) and the methyl (H-6',2.47) of IVb. This

Fig. 6. NOE difference spectra of TAN-1030 A.



NOE enhancements are shown by arrows.

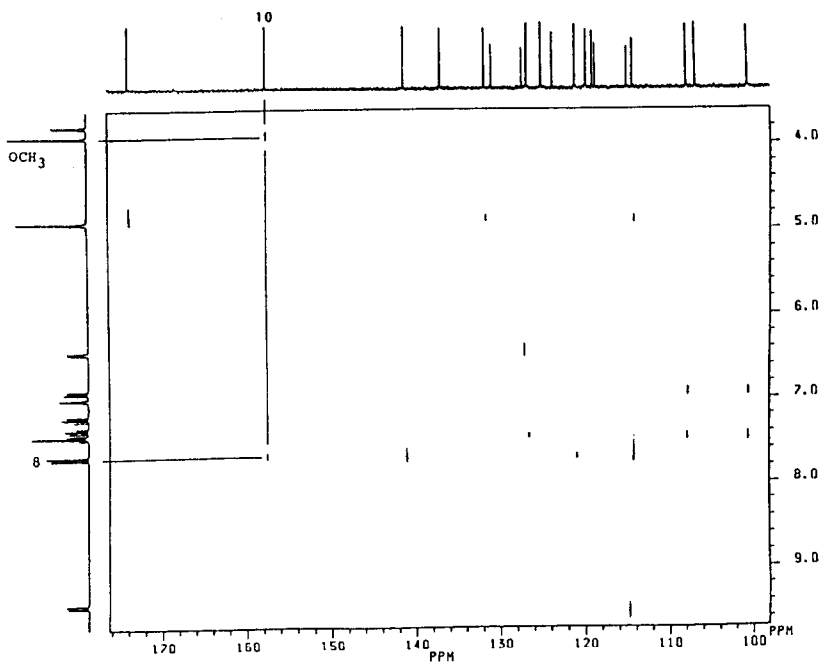
indicates the oxime stereochemistry. All of these data lead to partial structure V (Fig.4). The remaining quaternary carbon (C-12a,128.03) must constitute an aromatic ring with the other five quaternary carbons which are marked as * in V. Therefore, the structure of TAN-1030 A was determined to be as shown in Fig.1.

Structure determination of TAN-999

TAN-999 was obtained as pale yellow crystals with properties as follows: mp 221°C (dec), $[\alpha]_D$ +42° (c 0.50, dimethylformamide), molecular formula $C_{29}H_{28}N_4O_4$, IR (KBr) νmax 3430cm^{-1} (NH) and 1680 cm^{-1} (amide), UV λmax (MeOH) 245nm (ε31,200), 296 (60,300), 341 (19,500), 352 (sh,16,200) and 368 (11,000). The UV spectrum indicates that TAN-999 has a chromophore similar to the indolocarbazole moiety in staurosporine.

^1H and ^{13}C NMR data are summarized in Tables 1 and 2. The ^1H NMR spectrum of TAN-999 was similar to that of staurosporine, except the 1,2-disubstituted benzene moiety of staurosporine was replaced by a 1,2,4-trisubstituted benzene moiety (H-8,7.75, H-9,6.96 and H-11,7.46) and a methoxy signal (δ3.95) was observed. The difference in molecular formula (OCH_2) between TAN-999 and staurosporine confirmed the methoxy substitution of staurosporine. To determine the position of the methoxy substituent, ^1H-^1H

Fig. 7. COLOC spectrum of TAN-999 (J=4.2Hz).



COSY, ^{1}H-^{13}C COSY, refocussed INEPT and COLOC experiments were performed as in the case of TAN-1030 A. In the COLOC spectrum (J=4.2 Hz, Fig.7), a quaternary carbon (C-10, 157.49) was correlated with the methoxy protons (δ3.95) and the aromatic proton (H-8, Fig.8).

Instead of NOE differential experiments, a NOESY experiment was applied in the case of TAN-999. A cross peak was found between the methoxy protons and aromatic protons (H-9 and H-11) in the NOESY spectrum (Fig.9). Furthermore, an NOE was observed between the aromatic proton (H-11) and methyl protons (H-6',2.33). These data indicate that the methoxy group is bound to the 10-position. The NOESY spectrum also showed the correlation of H-7 (δ4.96) and H-8 and that of H-1' (δ6.53) and H-1 (δ7.27). Thus, the structure of TAN-999 was determined to be as shown in Fig.1.

Fig. 8. COLOC and NOESY data of TAN-999.

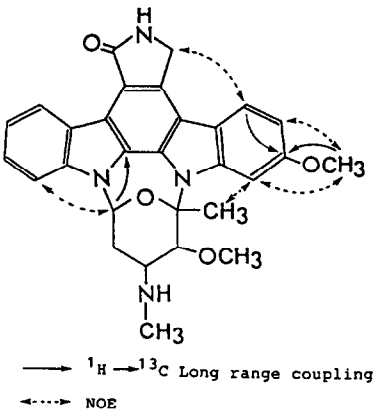
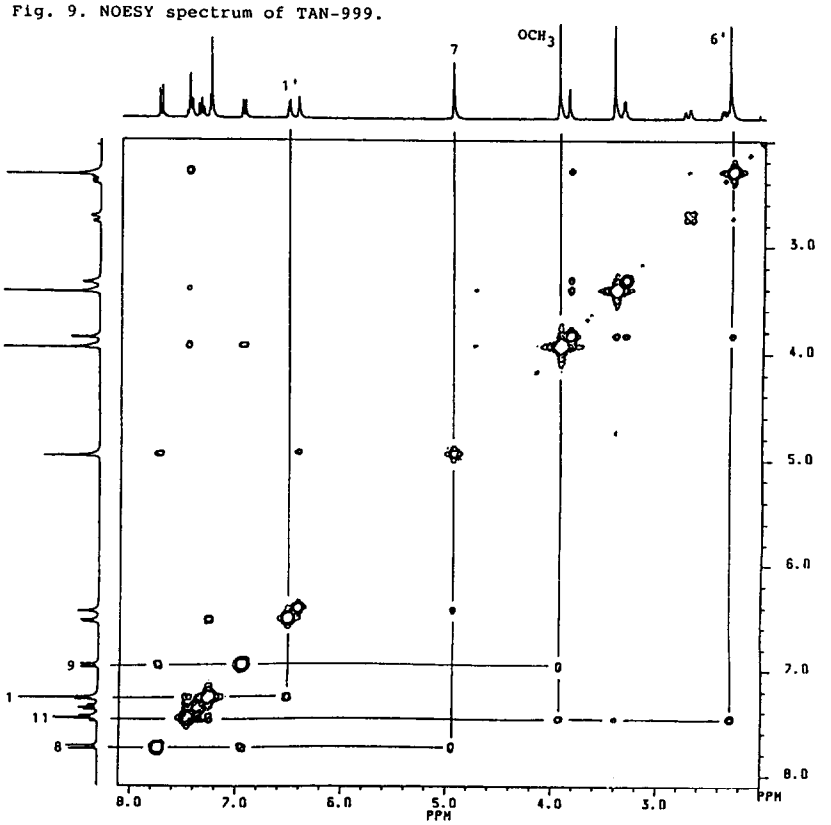


Fig. 9. NOESY spectrum of TAN-999.



Experimental

For the NMR experiments, approx. 0.1M solution of the sample was prepared. All NMR experiments were performed using the standard Bruker microprograms on a Bruker AC-300 spectrometer at 24°C. For homonuclear experiments matrixes were 256 X 1K data points and for the heteronuclear 128 X 4K data points. The ^{1}H-^{13}C COSY experiments were run with the XHCORR program and $^{1}J_{CH}$ was set equal to 130 Hz. The refocussed INEPT experiments were performed using the INEPTRD program. The COLOC experiments were run using the COLOC program. The conditions of COLOC experiments were as follows: number of scans, 200; total measuring time, 16hr 20min in the case of J= 12.5Hz and 17hr 12min in the case of J=4.2Hz. The NOESY experiments and the NOE differential experiments were performed using the NOESY program and the NOEDIFF program, respectively.

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