Tetrahedron. Vol. 30, pp. 4105 to 4111. Pergamon Press 1974. Printed in Great Britain

ISOLATION AND STRUCTURE OF TRICHOTOMINE AND TRICHOTOMINE G_1

S. IWADARE,* Y. SHIZURI, K. SASAKI and Y. HIRATA
Chemical Institute, Nagoya University, Chikusa-ku, Nagoya, Japan

(*Received in Japan* 8 *June* 1974; *Received in the UK for publication* 19 *June* 1974)

Abstract—The new pigments, trichotomine (1) and trichotomine G_1 (12), which have a novel carbon skelton, have been isolated from the fruits of the plant *Clerodendron trichotomum* Thunb. On the basis of physical and chemical evidence, the structure of trichotomine (1)[1,2] was elucidated as an oxidative dimeric compound of 3 - oxo - 2,3 - 5,6 - tetrahydro - 11H indolizino[8,7-b]indole 5-carboxylic acid and its stereostructure containing the absolute configuration was further determined by means of an x-ray crystallographic analysis.[3] The structure of trichotomine G_1 (12), a N-β-D-glucopyranosyl trichotomine, is also based on spectral and chemical evidence. A N-β-D-glucopyranosyl indole derivative such as (12) is the first naturally occurring example.[4]

The isolation of blue pigments from the fruits of the plant *Clerodendron trichotomum* Thunb was first undertaken by Giakomo Bionda *et al.*,[5,6] but they did not succeed in obtaining blue pigments in a pure form. As the UV spectra of these materials are quite different from those of anthocyanin and biliverdin, great efforts were made to isolate the new pigments. In this paper, we wish to report the isolation and structures of trichotomine (1) and trichotomine G_1 (12).

The structure of trichotomine (1). Trichotomine (1, m.p. > 300°) has a molecular formula $(C_{15}H_{10}N_2O_3)_2$. As shown in the NMR spectrum, the signals corresponding to five protons are found in the aromatic region, one of which is singlet at δ 7·22, but the others are double doublets (Experimental). Furthermore, the NMR signals corresponding to each one proton indicate the presence of an ABX type proton linkage. Accordingly, trichotomine (1) must have the partial structures A and B.

The IR spectrum of trichotomine (1) suggests the presence of carboxyl groups. In fact, treatment of trichotomine (1) with CH_2N_2 in MeOH gave a methyl ester (2, m.p. 285–287°), having a molecular formula $(C_{16}H_{12}N_2O_3)_2$. The IR absorption band at 1745 cm^{-1} and the NMR signal at δ 3·64 (3H × 2, s) both can be assigned to carbomethoxyl groups. Furthermore, a broad singlet at δ 10·97 disappeared on addition of D_2O. The dimeric structure was based on its molecular weight determination (569 ± 10). In the light of this molecular weight, the NMR spectrum of 2 showed that only one half of the total proton signals were detectable. Therefore, trichotomine methyl ester (2) must have a symmetrical structure. The presence of two OH or two NH

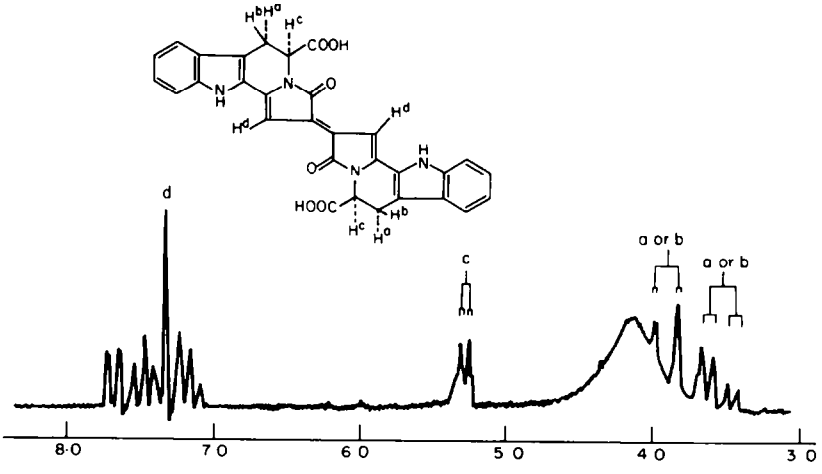
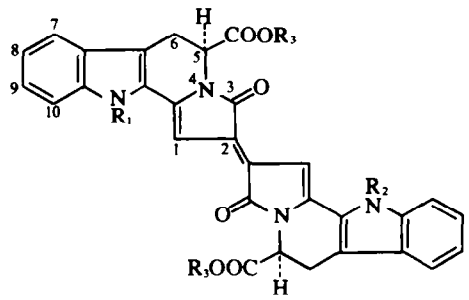
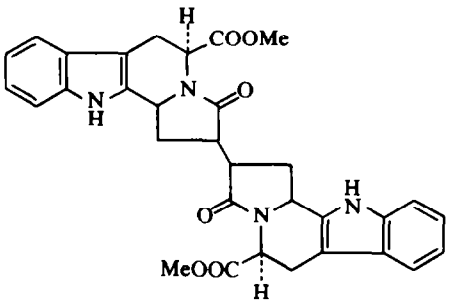


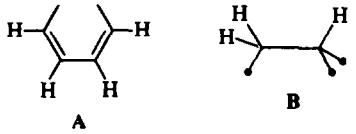
Fig 1. The NMR spectrum of trichotomine (1) (Cd_3COCD_3).



1: $R_1 = R_2 = R_3 = H$
2: $R_1 = R_2 = H$, $R_3 = Me$
3: $R_1 = H$, $R_2 = Ac$, $R_3 = Me$
4: $R_1 = R_2 = Ac$, $R_3 = Me$
8: $R_1 = R_2 = R_3 = Me$



5



A B

Fig 2.

groups can also be confirmed by acetylation which afforded a mixture of mono- and di-acetates in 32% and 58% yield, respectively.

Catalytic hydrogenation of trichotomine methyl ester (2) afforded a hexahydro-compound (5) in 35% yield. As shown below, particularly, the UV spectrum of compound (5) is almost superimposable to that of ibogamine (see the figure), indicating that (5) has an α,β-disubstituted indole nucleus in consideration of the NMR spectral data $\delta(CDCl_3)$ 7·0–7·15 (4H × 2, m, aromatic protons). Furthermore, the IR spectrum of 5 shows the presence of a 5-membered lactam. Accordingly, the IR absorption band at 1672 cm^{-1} in trichotomine methyl ester (2) must be assigned to the conjugated 5-membered lactam grouping. Further information of two partial structures [C and D] in 5 were obtained by the NMR spectral data coupled with the NMDR measurements: irradiation at δ 4·92 caused each multiplet at δ 2·31 and at δ 1·42 to collapse to double doublet, whereas each multiplet due to two protons of this methylene group became double doublet on irradiation at δ 3·33. Although 2 absorbed three molecules of hydrogen leading to the formation of 5, the area intensity of total protons in the latter appeared to increase by three protons as compared with that of the original methyl ester (2). Therefore, the two indole moieties can be connected to each other in such a manner as shown in C.

In conclusion, hexahydrotrichotomine methyl ester (5) must have the partial structure (6) or (7), From a biogenetic point of view, the latter that has a tryptophan flamework is more favourable than (6). In fact, the above conclusion was supported by the NOE measurements of N,N'-dimethyl-trichotomine methyl ester (8), in which the area intensity of the vinyl protons at δ 7·20 was enhanced by 15% on irradiation at δ 3·28 (>NMe) ($CDCl_3$-C_6D_6). Accordingly, the structure of trichotomine methyl ester must be represented by 9. When treated with 1N KOH–MeOH–Et_2O (1 : 1 : 1), the methyl ester (2) was readily converted into trichotomine. Thus, the structure (10) can be given to trichotomine.

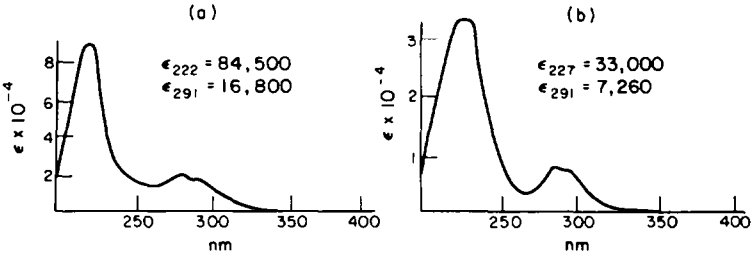


Fig. 3. The U. V. spectrum of (a) 5 and (b) ibogamine (MeOH).

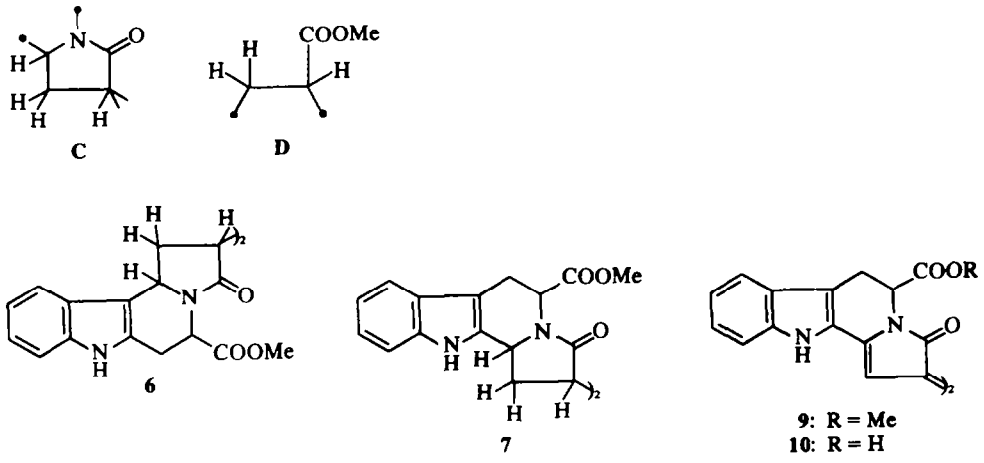


Fig 4.

The stereostructure of trichotomine (1), including the absolute configuration, was finally determined by an X-ray crystallographic analysis of the *p*-bromo-benzoate derivative (11)[3].

The structure of trichotomine G_1 (12). From the IR spectrum coupled with the NMR spectrum of trichotomine G_1 which has a doublet at δ 5·47 arising from an anomeric proton and complex signals at δ 3·0–4·0, it is considered that trichotomine G_1 (12) has a sugar portion. When hydrolysed with 3N methanolic HCl, trichotomine G_1 (12) yielded the methyl ester of its aglycone, trichotomine dimethyl ester (2), and D-glucose. Acetylation of trichotomine G_1 (12) afforded deep blue crystals (13, m.p. 174–176°) having a molecular formula $C_{44}H_{38}N_4O_{15}$. As shown in the NMR spectrum of trichotomine G_1 acetate (13), the sugar portion of this acetate (13) exhibits the signals corresponding to those of penta-O-acetyl β-D-glucose. The splitting of the anomeric proton at δ 5·70 (J = 7·2 Hz) in the NMR spectrum of 13 indicates the presence of a β-glycosidic bond. Consequently, 13 can be regarded as N-tetra-O-acetyl β-D-glucopyranosyl trichotomine. Thus, trichotomine G_1 (12) should be concluded as N-β-D-glucopyranosyl trichotomine.

The structure of N,N′-*di* (D-*glucopyranosyl*) *trichotomine* (14). From the IR spectrum coupled with the NMR spectrum of 14 which has complex signals at δ 3·0–4·0, it is considered that 14 has a sugar portion.

When hydrolysed with 3N methanolic HCl, this pigment yielded the methyl ester of its aglycone, trichotomine dimethyl ester (2), and D-glucose.

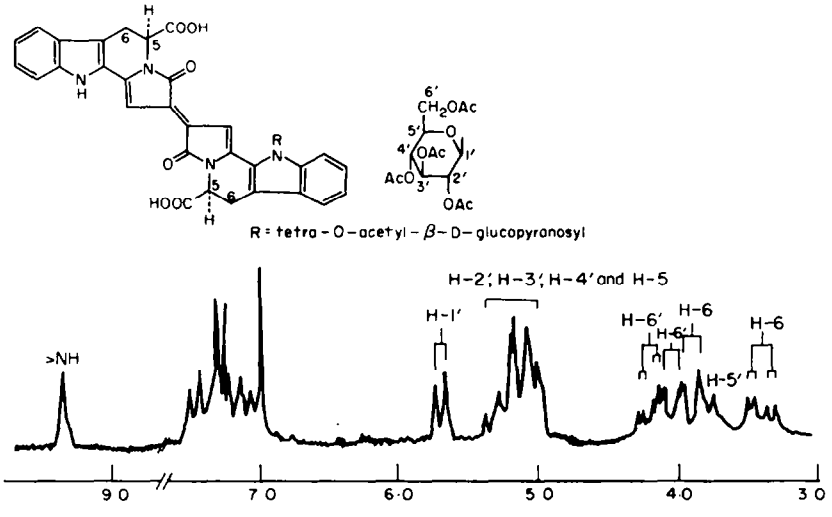
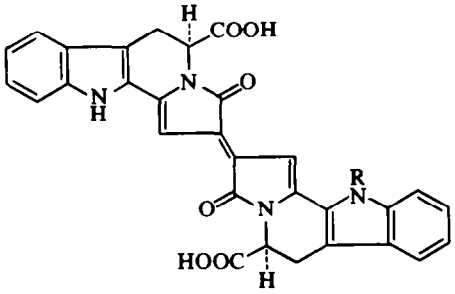
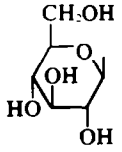


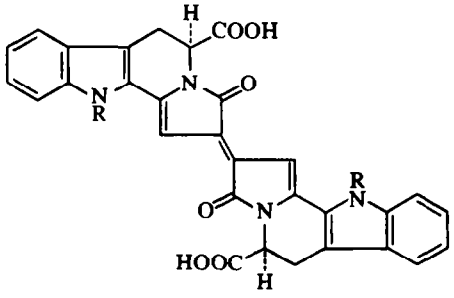
Fig 5. The NMR spectrum of trichotomine G_1 acetate (13) ($CDCl_3$).



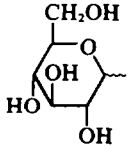
12: R = β-D-glucopyranosyl,
13: R = tetra-O-acetyl-β-D-glucopyranosyl



-β-D-glucopyranosyl



14: R = D-glucopyranosyl
15: R = tetra-O-acetyl-D-glucopyranosyl



-D-glucopyranosyl

Fig 6.

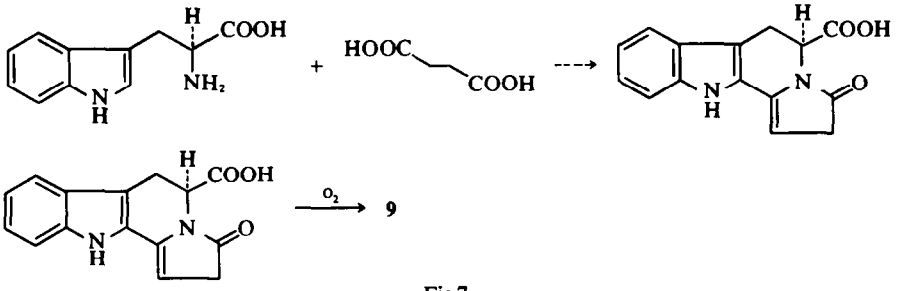


Fig 7.

Acetylation of **14** with Ac_2O-Py afforded a deep blue powder (**15**), the NMR spectrum of which showed the promient signals at δ 6·32 ($\frac{3}{5}$H, d, J = 3 Hz, H-1'), 5·72 ($\frac{4}{5}$H, d, J = 7·2 Hz, H-1') and 1·20–2·20 (3H × 8, s, $-OCOCH_3$). Therefore, this powder seems to be a mixture of the anomers of N,N'-di(tetra-O-acetyl-D-glucopyranosyl) trichotomine. Thus, the original blue powder (**14**) is considered to be a mixture of the anomers of N,N'-di(D-glucopyranosyl) trichotomine. The anomers with α-glycosidic bond may be produced from the β,-D-glucoside in the course of separation.[7]

From a structural point of view, these pigments present the first example of the new chromophore not hitherto found in this field. In addition, trichotomine G_1 and a mixture of the anomers of N,N'-di (D-glucopyranosyl) trichotomine are the first naturally occurring N-D-glucopyranosyl indole derivatives. Biogenetically, trichotomine can be regarded as an oxidative dimeric compound of 3-oxo-2,3-5,6-tetrahydro-11H indolizino[8,7-b]indole 5-carboxylic acid, which must be produced directly by the condensation of L-tryptophan and succinic acid, as shown in Fig 7. In fact, we have succeeded in the synthesis of trichotomine.[2]

EXPERIMENTAL

All m.ps were uncorrected. The UV spectra were measured with a Perkin Elmer Spectrophotometer. The IR spectra were recorded with a Nihon-Bunko IR-S Spectrophotometer. The CD spectra were taken on JASCO ORD/UV-5 Spectrophotometer. The NMR spectra were recorded with Varian Associates Spectrometers (A60 and HA-100); only promient peaks are cited; the

chemical shifts are given in ppm relative to internal TMS; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; coupling constants are given in Hz. The mass spectra were determined on a Hitachi RMU-6D mass spectrometer equipped with a direct inlet system and operating with an ionization energy of 70 ev. TLC analysis was performed on silica gel GF or silica gel PF (E. Merck, A. G., Germany). For column chromatography, Mallinckrodt silicic acid (100 mesh, Mallinckrodt, U.S.A.) was used.

Isolation. The blue fruits of the plant *Clerodendron trichotomum* Thunb (100 Kg) were collected in October and immersed in MeOH (120 *l*) for 2 weeks, and then filtered. The combined filtrates were concentrated under reduced pressure to *ca* 8·5 *l*. The remaining soln was washed with benzene (3 × 10*l*) to remove fatty acids, and then added to MeOH (33 *l*), to give a white ppt (1·5 kg). After removal of this ppt, the mother liquor was concentrated under reduced pressure to *ca* 4 *l*. To the aqueous soln was added MeOH (0·8 *l*), and then the remaining soln was allowed to stand at −25° overnight to yield a deep blue ppt (fraction A, *ca* 310 g), which were collected by decantation. The mother liquor was further concentrated under reduced pressure to *ca* 2·3 *l*, and then added to MeOH–CH_3COCH_3 (1:1, 7 *l*) to yield a white solid (1·2 kg). After removal of this solid, the filtrates were concentrated under reduced pressure to leave a blue residue (fraction B, *ca* 255 g). Fraction A (*ca* 50 g) was chromatographed on Sephadex LH-20 (500 g) using 10% aqueous MeOH as eluent, and then separated to 3 fractions: A-1 [10·5 g, elution volume (*ca* 1·5 *l*); this fraction did not contain blue pigments]; A-2 [24·9 g, elution volume (1·5–2·8 *l*); this fraction contained blue pigments in *ca* 0·2%]; A-3 [6·25 g, elution volume (2·8 *l*–6·1 *l*); this fraction contained blue pigments in 5·7%]. The remaining portion of fraction A was separated in the same way, and the total weight of A-3 was 46 g. The fraction (A-3) was again chromatographed on Sephadex LH-20 (500 g) using 10% aqueous MeOH as eluent to give two bands of blue pigments. The first-running fraction, A-3-1 [0·87 g, elution volume (0·74–1·30 *l*)], contained several blue pigments. In the slow-running fraction, A-3-2 [1·17 g, elution volume (1·30–2·05 *l*)], one blue pigment was mainly detected on TLC. The remaining portion of A-3 was also separated in the same way as described above to give two fractions [4·2 g (A-3-1) and 5·5 g (A-3-2)]. The fraction (A-3-2) was further separated by column chromatography on cellulose [CH_3COCH_3-C_6H_6 (5:1) as eluent], and then by precipitation method using MeOH-C_6H_6 to give an amorphous blue powder, trichotomine (**1**, 1·2 g), in a pure form. Fraction B was also separated in the same way as that of the fraction A to give trichotomine (**1**, 0·5 g), which showed the following physical data: m.p. >300°; ν_{max} (KBr) 1712(–COOH), 1650 and 1600 cm^{-1}; λ_{max} (MeOH) 660, 618, 353, 337 and 242 nm (ϵ, $6{\cdot}9\times10^4$, $6{\cdot}7\times10^4$, $3{\cdot}3\times10^4$, $2{\cdot}9\times10^4$ and $3{\cdot}1\times10^4$, respectively); δ (CD_3COCD_3) 7·62 (1H × 2, dd, J = 1·5, 7·8 Hz, aromatic protons), 7·45 (1H × 2, dd, J = 2·0, 7·6 Hz, aromatic protons), *ca* 7·3 (1H × 2, m, aromatic protons), 7·12 (1H × 2, dd, J = 2·0, 7·8 Hz, aromatic protons), 7·22 (1H × 2, s, vinyl protons), 5·20 (1H × 2, dd, J = 2·0, 7·0 Hz, $\rangle$C<u>H</u>$_X$–C<u>H</u>$_A$H$_B$–), 3·78 (1H × 2, dd, J = 2·0, 17·5 Hz, $\rangle$CH$_X$–C<u>H</u>$_A$H$_B$–) 3·42(1H × 2, d, J = 7·0, 17·5Hz, $\rangle$CH$_X$–C<u>H</u>$_A$H$_B$–) and *ca* 4·05 (2H × 2, br.s, exchangeable); CD (MeOH) $[\theta]_{375}$ + 21,900 and $[\theta]_{332}$ − 32,800; [Found: C, 67·21; H, 3·63; N, 10·17. $(C_{15}H_{16}N_2O_3)_2$ requires: 6, 67·66; H, 3·79; N, 10·52%].

The separation of trichotomine G_1 (**12**) *and a mixture of the anomers of* N,N′-di (D-*glucopyranosyl*) *trichotomine* (**14**).

The fraction A-3-1 (0·74 g)* was chromatographed on cellulose (70 g) using CH_3COCH_3–H_2O–C_6H_6 (150:×:50; × is gradually increased to 0, 3, 6, and 12) as eluent. Elution of blue pigments with CH_3COCH_3–H_2O–C_6H_6 (150:12:50, 0·88 *l*), followed by concentration under reduced pressure, afforded an amorphous blue powder (0·25 g). The remaining portion of A-3-1 was also separated in the same way as described above to give an amorphous blue powder (1·5 g), which was further chromatographed on Sephadex LH-20 (500 g, 10% aqueous MeOH as eluent) to give the further purified amorphous blue powder (0·62 g). This powder mainly contained two blue pigments that were further separated by TLC [CF_{254} (type 60), in CH_3COCH_3–H_2O–C_6H_6 (120:2:10)] to give **12** (0·18 g) and a mixture of the anomers of **14** (0·084 g).

Compound **12** shows the following physical data: m.p. >300°; ν_{max} (KBr) 3440 (–OH), 1760 (–COOH), 1660 and 1606 cm^{-1}; λ_{max} (MeOH) 658, 617, 350, 336 and 247 nm (ϵ, $3{\cdot}34\times10^4$, $3{\cdot}16\times10^4$, $1{\cdot}50\times10^4$, $1{\cdot}12\times10^4$ and $1{\cdot}52\times10^4$, respectively); δ (CD_3COCD_3–D_2O) 6·80–7·80 (8H, complex pattern, aromatic protons), 7·20 (1H × 2, s, vinyl protons), 5·47 (1H, d, J = 7·2 Hz, H-1′), 5·41 (1H × 2, dd, J = 2·0, 7·0 Hz, $\rangle$C<u>H</u>–CH$_2$–) and 3·0–4·0 (10 H, complex pattern); CD (MeOH) $[\theta]_{380}$ + 13,600 and $[\theta]_{330}$ − 24,800.

Compound **14** shows the following physical data: ν_{max} (KBr) 3450 (–OH), 1760 (–COOH), 1659 and 1603 cm^{-1}; λ_{max} (MeOH) 650, 610, 347, 334 and 234 nm (ϵ, $2{\cdot}10\times10^4$, $1{\cdot}94\times10^4$, $1{\cdot}10\times10^4$, $1{\cdot}04\times10^4$ and $1{\cdot}53\times10^4$, respectively); CD (MeOH) $[\theta]_{380}$ + 10,900 and $[\theta]_{330}$ − 19,100.

Trichotomine dimethyl ester (**2**). To a soln of **1** (50 mg) in MeOH was added an ethereal soln of CH_2N_2 (10 ml). The mixture was kept at room temp for 1 hr, and then concentrated under reduced pressure to leave an amorphous blue powder. Crystallization from MeOH gave needles (40 mg); m.p. 285–287°; ν_{max} ($CHCl_3$) 1745 (–COOMe), 1672 and 1606 (vs) cm^{-1}; λ_{max} ($CHCl_3$) 658, 620, 351, 340 and 245 nm (ϵ, $7{\cdot}0\times10^4$, $6{\cdot}1\times10^4$, $3{\cdot}4\times10^4$, $3{\cdot}0\times10^4$ and $3{\cdot}2\times10^4$, respectively); δ (CD_3COCD_3) 10·97 (1H × 2, s, exchangeable) and 3·64 (3H × 2, s, –COOMe); (Found: C, 68·07; H, 4·06; N, 9·67. $(C_{16}H_{12}N_2O_3)_2$ requires: C, 68·56; H, 4·32; N, 10·00%).

Acetylation of trichotomine dimethyl ester (**2**). A soln of **2** (40 mg) in Ac_2O (0·035 ml-Pyridine (10 ml) was kept at 80° for 30 min, and then concentrated under reduced pressure using C_6H_6 as azeotropic solvent to give an amorphous powder, which showed two spots on analytical TLC plate. The lower spot corresponds to **3**, and the upper one to **4**. These two compounds were separated by preparative TLC (silica gel PF_{254}, Merck) using $CHCl_3$–MeOH (15:0·1) as solvent system. Elution with MeCOMe gave an amorphous powder from the lower fraction, which was crystallized from MeOH to afford plates (**3**, 14 mg) m.p. 239–241°. From the upper fraction. an amorphous powder was also obtained, which was crystallized from MeOH to afford plates (**4**, 28 mg) m.p. 246–248°. (**3**): ν_{max} (KBr) 1735, 1715, 1668 and 1580 cm^{-1}; λ_{max} ($CHCl_3$) 655, 610, 350 and 243 nm (ϵ, $4{\cdot}44\times10^4$, $3{\cdot}97\times10^4$, $2{\cdot}30\times10^4$ and $2{\cdot}78\times10^4$, respectively); δ

($CDCl_3$) 2·70 (3H, s, $\rangle NCOCH_3$) and 9·40 (1H, s, exchangeable); (Found: C, 67·29; H, 4·10; N, 8·98. $C_{34}H_{36}N_4O_7$ requires: C, 67·77; H, 4·35; N, 9·30%), (4): ν_{max} (KBr) 1744, 1674 and 1584 cm^{-1}; λ_{max} ($CHCl_3$) 655, 612, 355 and 244 nm (ϵ, $4·32\times10^4$, $4·04\times10^4$, $2·63\times10^4$ and $2·69\times10^4$); δ ($CDCl_3$) 2·79 (3H×2, s, $\rangle NCOCH_3$); (Found: C, 67·62; H, 4·69; N, 8·29. $C_{36}H_{28}N_4O_8$ requires: C, 67·07; H, 4·38; N, 8·69%).

Catalytic hydrogenation of trichotomine dimethyl ester (2). Catalytic hydrogenation of 2 (40 mg) in AcOEt–MeOH (1:1, 110ml) was carried out over PtO_2 (10 mg) at room temp for 5 hr. After filtration of the catalyst, the solvent was removed under reduced pressure to give a white solid, which showed four spots on analytical TLC plate, one of which was a main spot. This main product was easily separated by preparative TLC (silica gel PF_{254}, Merck) using $CHCl_3$–MeOH (15:1) as solvent system to give a white powder of 5 (14 mg), which was not crystallized but showed one spot on analytical TLC plate. (5): *m/e* 566 (M^+ for $C_{32}H_{30}N_4O_6$); ν_{max} ($CHCl_3$) 3420 ($\rangle NH$), 2900, 1743, 1689 (5-membered lactam) and 1603 (w) cm^{-1}; λ_{max} (MeOH) 222, 280 and 291 nm (ϵ, $8·45\times10^4$, $1·97\times10^4$ and $1·68\times10^4$, respectively); δ ($CDCl_3$) 7·70 (1H×2, s, $\rangle NH$), 7·0–7·15 (4H×2, m, aromatic protons), 4·92 (1H×2, dd, 7·6, 9·4 Hz, $\rangle CH$–CHH–CH), 3·33 (1H×2, dd, J = 7·0, 7·6 Hz, $\rangle$CH–CHH–CH), 2·31 (1H×2, m, $\rangle$CH–CHH–CH), 1·42 (1H×2, m, $\rangle$CH–CHH–CH), 4·32 (1H×2, dd, J = 5·0, 7·5 Hz, $-CH_X$–CH_AH_B–) 3·30 (1H×2, dd, J = 7·5, 16·0 Hz, $\rangle CH_X$–CH_AH_B–), 3·01 (1H×2, dd, J = 5·0, 16·0 Hz, $\rangle CH_X$–CH_AH_B–) and 3·74 (3H×2, s, –COOMe).

N,N'-dimethyl trichotomine methyl ester (8). To a soln of 2 (30 mg) and NaH (10 mg) in anhyd DMF (10 ml was added MeI (21 mg), and the resulting soln was kept at room temp for 15 min with stirring. The soln was poured into sat NH_4Cl aq and then extracted with AcOEt. The extracts were washed well with water, and then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave an amorphous powder, which was recrystallized from AcOEt–MeOH (1:1) to give needles of 8 (24 mg); m.p. 287–288°; ν_{max} ($CHCl_3$) 1749, 1675 and 1592 cm^{-1}; λ_{max} ($CHCl_3$) 667, 622, 355, 340 and 242 nm (ϵ, $7·29\times10^4$, $5·78\times10^4$, $2·99\times10^4$, $2·71\times10^4$ and $3·19\times10^4$, respectively); δ ($CDCl_3$–C_6D_6) 7·20 (1H×2, s, vinyl protons) and 3·28 (3H×2, s, $\rangle NMe$); (Found: C, 69·07; H, 4·80; N, 9·52. $C_{34}H_{28}N_4O_6$ requires: C, 68·56; H, 4·70; N, 9·13%).

N,N'-Di (p-bromo-benzoyl) trichotomine methyl ester (11). To a soln of 2 (31 mg) and NaH (12 mg) in anhyd THF (7 ml) was added a soln of *p*-bromo benzoyl chloride (12 mg) in anhyd THF (0·2 ml). The mixture was kept at room temp for 10 min with stirring, and then concentrated under reduced pressure to give a syrup to which H_2O was added. The mixture was extracted with benzene (25 ml×3). The combined extracts were washed with sat NaCl aq, and then dried over $MgSO_4$.

Removal of the solvent under reduced pressure gave an amorphous powder, which was separated by column chromatography on silica gel (12 g) and eluted with C_6H_6–$CHCl_3$ (2:1) to give an amorphous powder. This powder was crystallized from AcOEt to give needles of 11 (39 mg); m.p. 272–273°; ν_{max} ($CHCl_3$) 1746, 1680 and 1586 cm^{-1}; δ ($CDCl_3$) 7·62 (4H×2, s, aromatic protons of *p*-bromo-benzoyl groups), 7·10–7·60 (4H×2, complex pattern, aromatic protons) and 6·37 (1H×2, s, vinyl protons); (Found: C, 59·43; H, 2·92; N, 5·96. $C_{46}H_{30}N_4O_8Br_2$ requires: C, 59·62; H, 3·27; N, 6·00%).

Hydrolysis of trichotomine dimethyl ester (2). A soln of 2 (29 mg) in 1N KOH–MeOH–ether (1:1:1, 60 ml) was kept at room temp for 3 hr. Addition of ether (60 ml) yielded two phases. The aqueous layer was separated, and then acidified with conc HCl and extracted with EtOAc. The extracts were washed with sat NaCl aq and then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave an amorphous blue powder, which was further purified by precipitation method using MeOH–C_6H_6 to afford an amorphous blue powder, trichotomine (I, 16 mg), which was confirmed by its spectral data (IR, UV and NMR) and TLC.

Hydrolysis of trichotomine G_1 (12) *with* 3N *methanolic* HCl. A soln of 12 (5 mg) in 3N methanolic HCl (2 ml) was heated under reflux for 1 hr, and then concentrated under reduced pressure. The remaining aqueous soln was extracted with EtOAc (5 ml×3), and then the combined extracts were washed with sat NaCl aq several times, followed by concentration under reduced pressure, afforded an amorphous blue powder, which was crystallized from MeOH to give needles (m.p. 284–287°). These needles were identified as 2 by m.p., IR and UV spectra, and TLC. In the above aqueous phase, the presence of glucose was demonstrated by paper chromatographic analysis and comparison with an authentic specimen, in n-BuOH–EtOH–H_2O (13:8:4, Toyo, No. 51A, descending development). The D-configuration of glucose was further confirmed by the ultra micro method of E.C. 1.1.3.4. Worthington, D-glucose oxidase (A-niger) and Park-Johnson method (determination of reducing power test).

Acetylation of trichotomine G_1 (12). To a soln of 12 (24 mg) in pyridine (5 ml) was added Ac_2O (0·07 ml). The mixture was kept at room temp overnight, and then concentrated under reduced pressure using C_6H_6 as azeotropic solvent to give an amorphous powder. Separation of this powder by preparative TLC [GF_{254} (type 60), in AcOEt–C_6H_6 (5:2)] afforded an amorphous powder, which was crystallized from 2% aqueous MeOH to give needles of 13 (15 mg): m.p. 174–176°; ν_{max} ($CHCl_3$) 1763 (broad, –$OCOCH_3$ and –COOH), 1675 and 1604 cm^{-1}; λ_{max} ($CHCl_3$) 658, 612, 347, 335 and 244 nm (ϵ, $4·15\times10^4$, $3·50\times10^4$, $1·75\times10^4$, $1·58\times10^4$ and $1·87\times10^4$, respectively); δ ($CDCl_3$) 9·34 (1H, s, $\rangle NH$), 7·0–7·6 (8H, complex pattern, aromatic protons), 7·32 (1H, s, vinyl proton), 6·99 (1H, s, vinyl proton), 5·70 (1H, d, J = 7·2 Hz, H-1'),* 5·13 (1H×2, dd, J = 2·0, 7·0 Hz, $\rangle CH$–CH_2–), 4·90–5·40 (3H, complex pattern, H-2', H-3', H-4'), 4·20 (1H, dd, J = 4·2, 11·0 Hz, H-6'), 4·07 (1H, dd, J = 2·0, 11·0 Hz, H-6'), *ca* 3·80 (1H, m, H-5'), 3·89 (1H×2, dd, J = 2·0, 14·0 Hz, $\rangle$CH–CH_2–), 3·44 (1H×2, dd, J = 7·0, 14·0 Hz,

*These protons (H-1', H-2', H-3', H-4', H-5' and H-6') show the carbon-bonded protons of the glucose moiety.

$\diagdown$CH–CH$_2$–), 2·02 (3H × 2, s, –OCOCH$_3$), 1·95 (3H, s, –OCOCH$_3$) and 1·84 (3H, s, –OCOCH$_3$); CD (CHCl$_3$) $[\theta]_{375}$ + 15,400 and $[\theta]_{335}$ − 27,800; (Found: C, 60·75; H, 4·44; N, 6·03. $C_{44}H_{38}N_4O_{15}$ requires: C, 61·24; H, 4·45; N, 6·49%).

Hydrolysis of a mixture of the anomers of N,N'-*di* (D-*glucopyranosyl*) *trichotomine* (**14**). A soln of **14** (5 mg) in 3N methanolic HCl (2 ml) was heated under reflux for 1 hr, and then concentrated under reduced pressure. Work up in the same way as that of **12** yielded **2** and D-glucose. These two compounds were confirmed in the same way as shown above (see the hydrolysis of trichotomine G_1).

Acetylation of a mixture of the anomers of N,N'-*di* (D-*glucopyranosyl*) *trichotomine* (**14**). To a soln of **14** (23 mg) in pyridine (1·5 ml) was added Ac_2O (0·05 ml). The mixture was kept at room temp overnight, and then concentrated under reduced pressure using C_6H_6 as azeotropic solvent to give an amorphous powder. Separation of this powder by preparative TLC [GF_{254} (type 60), in AcOEt–C_6H_6 (5:2)] to give an amorphous blue powder of **15** (22 mg); ν_{max} ($CHCl_3$) 1763 (–OCOCH$_3$ and –COOH), 1673 and 1605 cm^{-1}; λ_{max} ($CHCl_3$) 658, 617, 350, 336 and 247 nm (ϵ, 2·83 × 10^4, 2·40 × 10^4, 1·52 × 10^4, 1·44 × 10^4 and 1·78 × 10^4, respectively); δ ($CDCl_3$) 6·32 ($\frac{1}{3}$ H, d, J = 3 Hz, H-1'), 5·72 ($\frac{2}{3}$ H, d, J = 7·2 Hz, H-1'), 2·18, 2·12, 2·09, 2·02, 1·99, 1·96 (3H, s, –OCOCH$_3$, respectively) and 2·03 (3H × 2, s, –OCOCH$_3$).

Acknowledgement—The authors are grateful to Professor S. Suzuki and Dr. M. Tsuji (Department of Chemistry, Faculty of Science, Nagoya University) for identification of D-glucose.

REFERENCES

[1]S. Iwadare, Y. Shizuri, K. Sasaki and Y. Hirata, *Tetrahedron Letters* 1051 (1974)
[2]S. Iwadare, Y. Shizuri, K. Yamada and Y. Hirata, *Ibid.* 1177 (1974)
[3]K. Sasaki, S. Iwadare and Y. Hirata, *Ibid.* 1055 (1974)
[4]E. Walton, F. W. Holly and S. R. Jenkins, *J. Org. Chem.* **33** 192 (1968)
[5]Giacomo Bionda, *Ann. Chim. Applicata.* **36**, 210 (1946)
[6]Victor Plouvier, *C. R. Acad. Sci., Paris* **231**, 1546 (1950)
[7]E. L. Jacobson, M. K. Jacobson and C. Bernofsky, *J. Biol. Chem.* **248** No. 22 7891 (1973)