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# THE STRUCTURES OF EUMITRINS $A_1$, $A_2$ AND B

## THE YELLOW PIGMENTS OF THE LICHEN, *USNEA BAYLEYI*(STIRT.)ZAHLBR.

DUN-MEI YANG, N. TAKEDA,* Y. IITAKA, U. SANKAWA and S. SHIBATA†
Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo

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**Abstract**—The yellow pigments named eumitrins $A_1$, $A_2$ and B were isolated from the lichen, *Usnea bayleyi*(Stirt.)Zahlbr. The modified bixanthone structures of these compounds were deduced mainly from their spectral data, and their absolute structures (**2a**, **3a** and **4a**) were established on the basis of X-ray crystallographic analysis of a transformed product (**6**) of tribromo-eumitrin B (**5**).

The occurrence of a characteristic pigment named eumitrin was first observed by Asahina[1] in the lichen *Usnea bayleyi*(Stirt.)Zahlbr. using the microscopical method. Nuno[2] isolated eumitrins A and B from the benzene extracts of the lichen along with (+) usnic acid, protolichesterinic acid, barbatic acid, norstictic acid, thamnolic acid, protocetraric acid, zeorin and an unknown red pigment. Following the above work, we found that the eumitrin A fraction can be separated into $A_1$ and $A_2$ by careful recrystallization. The present paper reports the chemical structures of eumitrins $A_1$, $A_2$ and B.

The dried lichen thalli of *Usnea bayleyi* was benzene extracted and fractionated as follows:

According to physical properties and spectral data, it was suggested that eumitrins $A_1$, $A_2$ and B are homologous compounds structurally related to secalonic acid A (= ergochrome AA(2,2')) (**1**). Secalonic acid A which was first isolated from ergot (sclerotium of *Claviceps purpurea* Tulasne)[3,4] and then from *Aspergillus ochraceus* Wilhelm[5] and the lichens, *Parmelia entotheiochroa* Hue[6] and *Cetraria ornata* Müll.[7], was initially formulated as a 4,4'-dimer of a modified xathone or secoanthraquinone, but recently was revised to a 2,2'-dimer.[8,9]

The UV-spectral curves of the eumitrins showed the presence of a 5-hydroxychromanone nucleus having an exocyclic double bond similar to secalonic acid A.

The IR spectra of the eumitrins revealed the presence of the following: hydrogen bonded hydroxyl, hydrogen bonded ketone, acetoxyl, carbomethoxyl and allylic methylene groups and an aromatic nucleus.

The brown red colouration of eumitrin $A_1$ with $FeCl_3$ suggested the presence of an enolized $\beta$-diketone system by analogy with secalonic acid A.

Methylation of eumitrin $A_1$ with $CH_2N_2$ afforded a dimethyl ether, m.p. 150°, which gave a 5-hydroxychromone like olive-green ferric reaction. The ferric reactions of eumitrins $A_2$ and B showed colouration between that given by eumitrin $A_1$ and 5-hydroxychromone.

The mass spectrum of eumitrin $A_1$ has a base peak at $m/e$ 621 ($M^+$—$COOCH_3$) corresponding to

*née Kobayashi.
†The author to whom inquiries should be addressed.

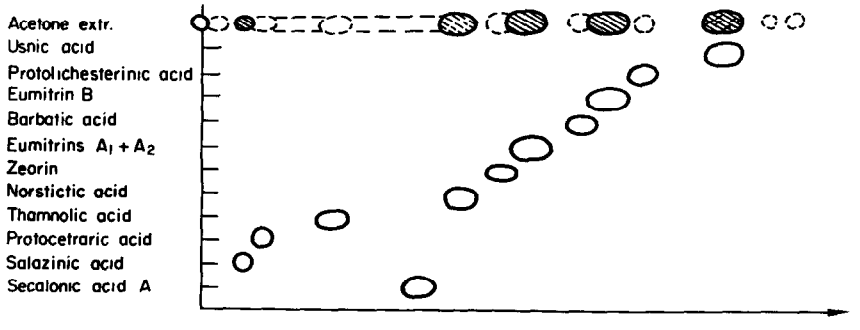
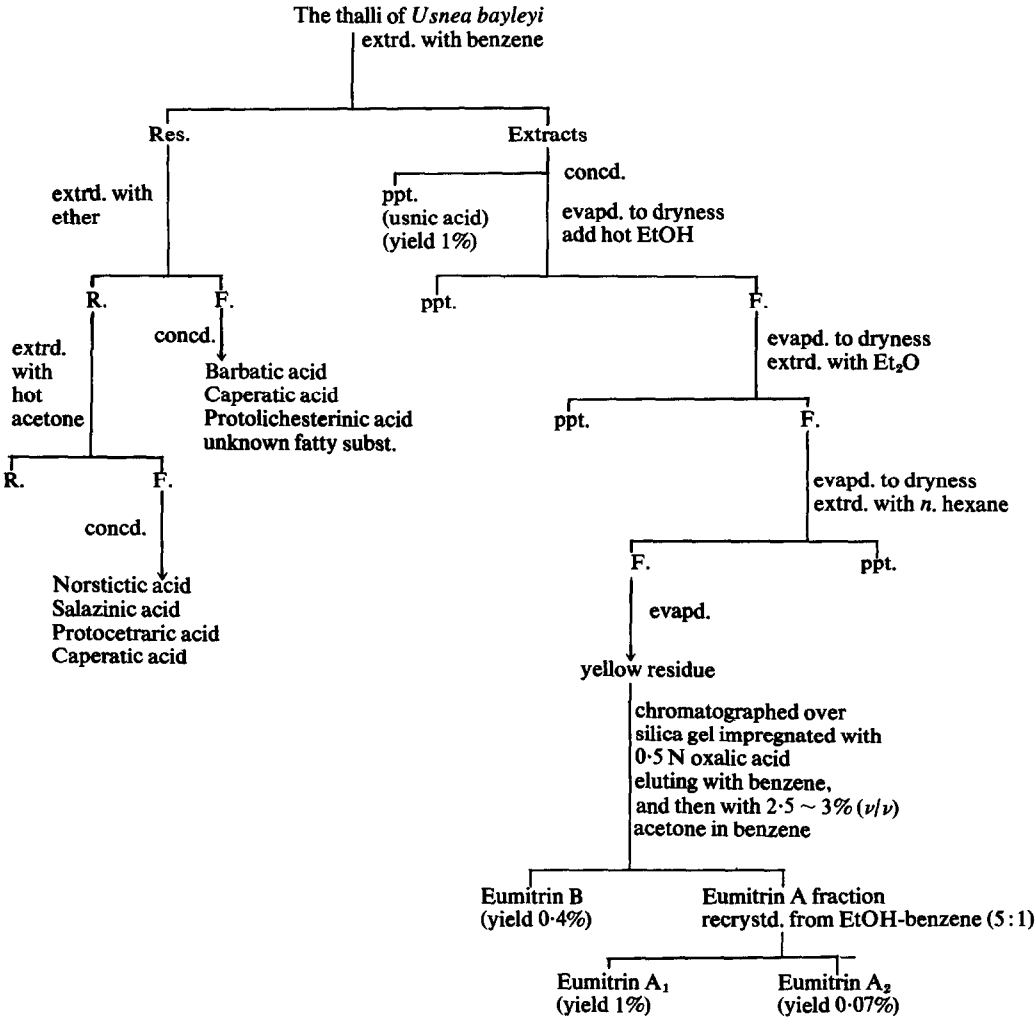



Fig 1. TLC of the acetonic extracts of *Usnea bayleyi*.




The TLC of acetonic extracts of *Usnea bayleyi* was illustrated in Fig. 1. Eumitrins $A_1$ and $A_2$ could not be separated on TLC.

Chart 1.

Table 1. The physical properties of eumitrins $A_1$, $A_2$ and B in comparison with those of secalonic acid A

| | Eumitrin $A_1$ | Eumitrin $A_2$ | Eumitrin B | Secalonic acid A (Ergochrome AA (2·2′)) |
|---|---|---|---|---|
| Colour and Cryst. form. | yellow plates | yellow needles | yellow prisms | yellow needles |
| m.p. | 249–252° | 216–220° | 238–240° | 260° |
| $[\alpha]_D$ | −52·4° (dioxan) | −76·7° (dioxan) | −33·3° (dioxan) | −73° ($CHCl_3$) |
| $M^+$ Obs. | 680·170 | 666·192 | 666 | 638 |
| $M^+$ Calc. | 680·174 | 666·195 | — | — |
| Mol. formula | $C_{34}H_{32}O_{15}$ | $C_{34}H_{34}O_{14}$ | $C_{34}H_{34}O_{14}$ | $C_{32}H_{30}O_{14}$ |
| $FeCl_3$ | red brown | greenish red brown | greenish red brown | red brown |
| Gibbs react. | — | — | — | — |

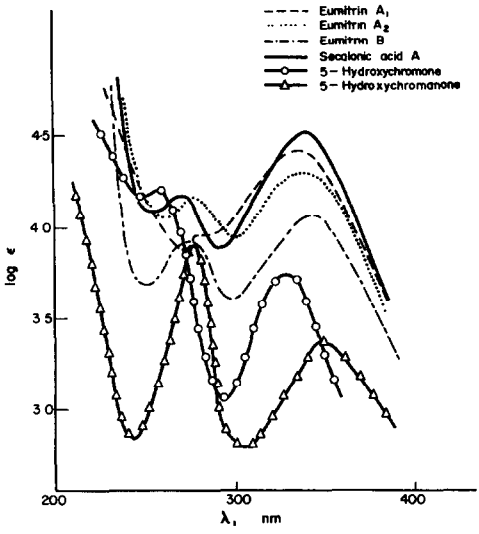



Fig 2.

a peak at *m/e* 607 (eumitrins $A_2$ and B) and at *m/e* 579 (secalonic acid A). The peak at *m/e* 561 given by eumitrin $A_1$ showed abstraction of $COOCH_3$ and $CH_3COOH$ from the molecular ion ($M^+$), and corresponding fragmentation patterns were observed for eumitrins $A_2$ and B at *m/e* 547. The mass spectra of the eumitrins revealed the pigments' dimeric nature and suggested that eumitrins $A_2$ and B are stereoisomers.

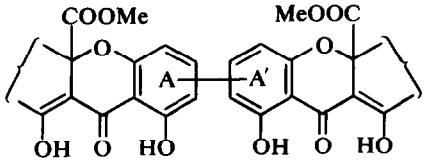
The NMR spectra of eumitrins $A_1$, $A_2$ and B (Table 5) also indicate their dimeric nature and asymmetric structure. The signal at δ 4·16 in the NMR spectrum of eumitrin $A_1$ was shifted to lower field (δ 5·62) in its monoacetate and at δ 5·65 in its peracetate. Therefore, it was assigned to the proton attached to the carbon bearing a secondary hydroxyl.

The low hydroxyl signals of eumitrin $A_1$ at δ 13·76 and 11·82 were assigned to the hydrogen bonded enolic hydroxyls, as they are not present in eumitrin $A_1$ dimethyl ether, while the signals of two phenolic hydroxyls of eumitrin $A_1$ at δ 11·18 and 11·52 shifted to δ 12·50 and 12·95, respectively, on methylation of the enolic hydroxyls due to the stronger hydrogen bond formation of the phenolic hydroxyls.

In the NMR spectra of eumitrins $A_2$ and B, only one enolic hydroxyl signal was detected at δ 13·98 and δ 13·65, respectively, while a methine proton signal was observed at δ 2·97 and δ 3·30, respectively.

Thus the partial structure of eumitrin $A_1$ is formulated as (**a**), and those of eumitrins $A_2$ and B as (**b**).

The NMR spectral patterns of eumitrins $A_2$ and B resemble each other very closely, except the coupling pattern of the proton attached to the carbon bearing the acetoxyl.




1 Me
either in A or A' ring

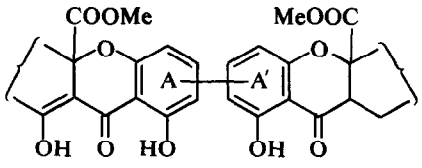
**a**

Table 2. UV spectra of eumitrins $A_1$, $A_2$ and B in comparison with that of secalonic acid A ($\lambda_{max}^{dioxan}$ nm (log ε))

| Eumitrin $A_1$ | Eumitrin $A_2$ | Eumitrin B | Secalonic Acid A |
|---|---|---|---|
| 272 (3·99) | 262 (4·17) | 253 (3·79) | 254 (4·20) |
| 281 (4·02) | 278 (4·26) | 275 (4·02) | 268 (4·25) |
| 286 (4·02) | 298 (4·05) | 295 (3·72) | 288 (4·00) |
| 334 (4·51) | 335 (4·39) | 336 (4·17) | 338 (4·61) |

Table 3. IR spectra of eumitrins $A_1$, $A_2$ and B in comparison with that of secalonic acid A ($\gamma_{max}^{KBr}$ $cm^{-1}$)

| | Eumitrin $A_1$ | Eumitrin $A_2$ | Eumitrin B | Secalonic Acid A |
|---|---|---|---|---|
| H-bonded OH | 3480 | 3480 | 3520 | 3480 |
| Acetate C=O | 1755 (sh) | 1755 (sh) | 1755 (sh) | — |
| —COOMe | 1745 | 1745 | 1740 | 1742 |
| H-bonded Keto C=O | 1615 | 1615 | 1610 | 1608 |
| Aromatic | 1580 | 1585 | 1580 | 1588 |
| | 1560 | 1565 | 1560 | 1560 |
| Allylic methylene | 1440 | 1435 | 1435 | 1435 |




1 Me
either in A or A' ring
b

This proton in eumitrins $A_2$ and B was assigned to the signal at δ 5·02 and 5·43, respectively, (not affected by acetylation). The half band width ($W_{1/2}$) of the δ 5·02 signal in eumitrin $A_2$ is *ca.* 12 Hz, whereas that given by eumitrin B at δ 5·43 is *ca.* 5 Hz as is the corresponding signal at δ 5·53 in eumitrin $A_1$. It has, therefore, been concluded that eumitrins $A_2$ and B are epimers at the secondary acetoxyl grouping, and eumitrins $A_1$ and B possess the same configuration at this position.

A double resonance experiment ($d_6$-acetone) of eumitrin B irradiating the proton at δ 1·84 made the triplet-like signal at δ 5·43 a sharp singlet. Thus an acetoxyl group is adjacent to a methylene and an adjacent carbon is quaternary, where a carbomethoxyl grouping is attached to give a deshielding effect to the proton signal absorbing at δ 5·43. Similar coupling effects were observed in the corresponding functional groups in eumitrin $A_1$. The differences observed in the coupling patterns of the proton attached to the carbon bearing acetoxyl in eumitrins $A_1$, $A_2$ and B can be elucidated by the following partial configurations. (**c** ~ **e**).

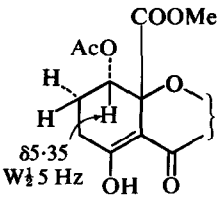
The proton giving the signal at δ 5·35 and δ 5·43 in the NMR spectra of eumitrins $A_1$ and B, respectively, bisects the dihedral angle of the adjacent methylene protons to result in giving $J_{HH'}$ 5 Hz (calc. $J_{HH'}$ 4–6 Hz), whereas the proton resonating at δ 5·02 of eumitrin $A_2$ forms a *trans* diaxial conformation with respect to one of the protons of the adjacent methylene and also with the adjacent carbomethoxyl group to result in a higher chemical shift and a large coupling constant, $W_{1/2}$ *ca.* 12 Hz (calc. $J_{HH'}$ *ca.* 10 Hz). The secondary hydroxyls of eumitrins $A_1$, $A_2$ and B must be located in a position adjacent to the secondary methyl groups, since irradiation of the proton (δ 2·20) attached to the carbon bearing methyl showed a coupling with the

Table 4. Mass spectra of eumitrins $A_1$, $A_2$ and B and secalonic acid A

| | Eumitrin $A_1$ | Eumitrin $A_2$ | Eumitrin B | Secalonic acid A |
|---|---|---|---|---|
| $M^+$ *m/e* | 680 (35%) | 666 (56%) | 666 (25%) | 638 (31%) |
| $M^+-59$ | 621 (100%) | 607 (100%) | 607 (100%) | 579 (100%) |
| $M^+-59-18$ | | | | 561 (10%) |
| $M^+-59-42$ | | 565 (35%) | 565 (38%) | |
| $M^+-59-18-60$ | | | | 501 (16·6%) |
| $M^+-59-60$ | 561 (23%) | 547 (22%) | 547 (22%) | |
| $M^+-59-60-60$ | 501 (9%) | 487 (12%) | 487 (24·5%) | |
| $M^+-59-42-96$ | | 469 (10%) | 469 (11%) | |
| $M^+-59-42-60$ | | 409 (6%) | 409 (10%) | |

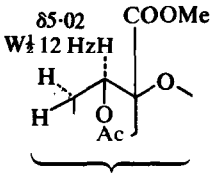
Table 5. The chemical shifts (δ) in the 100 MHz NMR spectra of eumitrins $A_1$, $A_2$ and B (in $CDCl_3$)

| | Eumitrin $A_1$ | Eumitrin $A_2$ | Eumitrin B |
|---|---|---|---|
| >CH—$CH_3$ | 1·18 (3H, d. *J* 6 Hz) | 1·17 (3H, d. *J* 6 Hz) | 1·22 (3H, d. *J* 6 Hz) |
| —OCO$CH_3$ | 1·85 (3H, s.) | 1·93 (3H, s.) | 1·88 (3H, s.) |
| arom. $CH_3$ | 2·07 (3H, s.) | 2·13 (3H, s.) | 2·06 (3H, s.) |
| >$CH_2$ / >C—H | 1·50–2·20 (*ca.* 3H, m.)<br>2·41–2·62 (*ca.* 4H, m.) | 1·50–2·55 (m.) | 1 40–2·65 (m.) |
| >C—H | — | 2·97 (1H, m.) | 3·30 (1H, m.) |
| —$COOCH_3$ | 3·69 (3H, s.) | 3·62 (3H, s.) | 3·68 (3H, s.) |
| | 3·76 (3H, s.) | 3·63 (3H, s.) | 3·78 (3H, s.) |
| >CH—OH | 4·16 (1H, br. s. $W_{1/2}$, 2·5 Hz) | 4·13 (1H, br. s. $W_{1/2}$, 2·5 Hz) | 4·17 (1H, br. s. $W_{1/2}$, 2·5 Hz |
| >CH—OAC | 5·35 (1H, tr. like $W_{1/2}$ *ca.* 5$H_2$) | 5·02 (1H, m. $W_{1/2}$, *ca.* 12 Hz) | 5·43 (1H, m. $W_{1/2}$, *ca.* 5 Hz) |
| arom. H | 6·47 (1H, s.) | 6·48 (1H, s.) | 6·45 (1H, s.) |
| arom. H (ortho) | 6·58<br>7·23 (2H, AB q. *J* 8 Hz) | 6·52<br>7·63 (2H, AB q. *J* 8 Hz) | 6·55<br>7·07 (2H, AB q. *J* 8 Hz) |
| Phenol. OH (H-bonded) | 11·18 (1H, s.) | 11·41 (1H, s.) | 11·39 (1H, s.) |
| | 11·52 (1H, s.) | 11·58 (1H, s.) | 11·56 (1H, s.) |
| enol. OH (H-bonded) | 13·76 (1H, s.)<br>13·82 (1H, s.) | —<br>13·98 (1H, s.) | —<br>13·65 (1H, s.) |



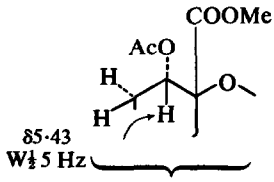

Eumitrin $A_1$

c




Eumitrin $A_2$

d

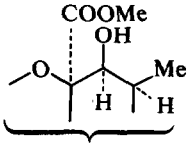



Eumitrin B

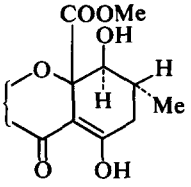
e

proton signal of >CH(OH) at δ 4·16, 4·13 and 4·17 (in $CDCl_3$), respectively. The corresponding signals were observed at δ 4·65–4·63 when measured in $d_5$-pyridine.

Thus eumitrins $A_1$, $A_2$ and B possess the following relative configurations (**f**) of these functional groups, corresponding to the configurations of the hydroaromatic portions of secalonic acid B whose signal of >CH(OH) appears at δ 4·55 with $W_{1/2}$ 3·5 Hz,[10] whereas the corresponding proton signal of secalonic acid A was given at δ 4·18 ($d_5$-pyridine) with an entirely different coupling pattern (d $J$ 10·5 Hz[3b]).

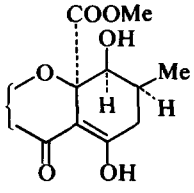



**f**




**g**

Secalonic acid A




**h**

Secalonic acid B

It has been proved by the above spectral data that the two halves of eumitrins are linked at their aromatic rings. As shown,[11] the proton located at the *para* position of a phenolic hydroxyl shows a down-field shift (0·25–0·4 ppm) on acetylation of the hydroxyl, whereas the proton located at the *ortho* or *meta* position of a hydroxyl gives no remarkable down-field shift (0·05–0·1 ppm). The acetylation of eumitrins $A_1$, $A_2$ and B caused a remarkable down-field shift of only one out of three aromatic protons in each compound.

Consequently, the biphenyl junction in eumitrins $A_1$, $A_2$ and B has been assigned to a 4,2′-linkage. From the biogenetic viewpoint, it would not be plausible to localize two C-methyl groups in one monomeric moiety of such modified bixanthone, while there still remains alternative possibilities of location of the ring involving an enol system.

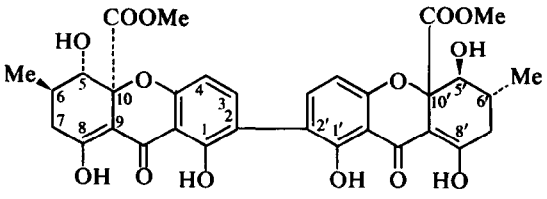
Thus eumitrin $A_1$ is now formulated as (**2a**), and eumitrins $A_2$ and B are represented by the alternative formulae (**3a**) or (**3a′**) and (**4a**) or (**4a′**), respectively. The final confirmation of the structures of eumitrin $A_1$ (**2a**), eumitrin $A_2$ (**3a**) and eumitrin B (**4a**) involving their absolute configurations has been performed by X-ray crystallographic analysis of a bromo derivative derived from eumitrin B.

Bromination of eumitrin B afforded a tribromo derivative, $C_{34}H_{31}O_{14}Br_3$, m.p. 225°, along with a small amount of a dibromo derivative, m.p. 183°, (separated by chromatography). The NMR spectrum of the tribromo derivative revealed that the two aromatic protons at the 2 and 4′ positions of eumitrin B have been substituted with bromine, and one bromine has been introduced into an angular position at $C_{(9')}$, since two aromatic proton signals and one enolic hydroxyl signal observed in the NMR spectrum of eumitrin B disappeared on bromination.

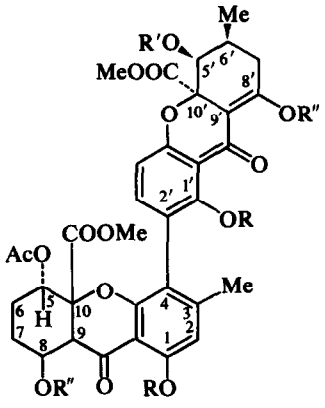
During the process of recrystallization from MeOH, tribromoeumitrin B was converted into an entirely different compound, $C_{34}H_{31}O_{14}Br_3$, m.p. 224–225°, whose IR spectrum (KBr) showed the

Table 6. Shift of the aromatic proton signals on acetylation of the phenolic hydroxyls ($CDCl_3$, δ ppm.)

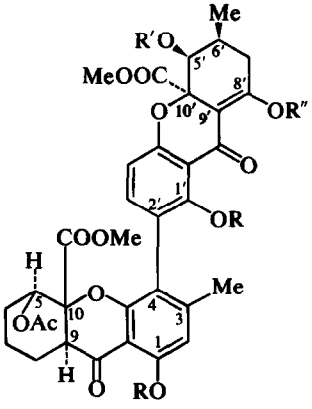
| | $C_{(2)}-H$ ($o$) | | $C_{(3')}-H$ ($m$) | | $C_{(4')}-H$ ($p$) | |
|---|---|---|---|---|---|---|
| Eumitrin $A_1$ | 6·47 | | 7·23 | | 6·58 | |
| | | Δ −0·07 | | Δ −0·08 | | Δ −0·38 |
| Peracetate | 6·54 | | 7·31 | | 6·96 | |
| Eumitrin $A_2$ | 6·48 | | 7·63 | | 6·52 | |
| | | Δ −0·09 | | Δ +0·04 | | Δ −0·48 |
| Peracetate | 6·57 | | 7·59 | | 7·00 | |
| Eumitrin B | 6·45 | | 7·07 | | 6·55 | |
| | | Δ −0·09 | | Δ −0·09 | | Δ −0·41 |
| Peracetate | 6·54 | | 7·16 | | 6·96 | |



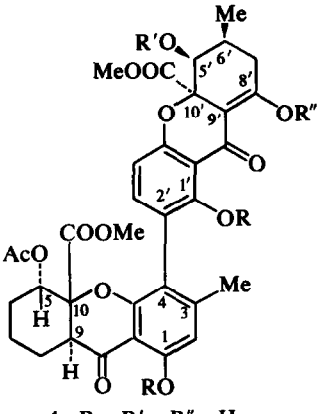
1
Secalonic acid A



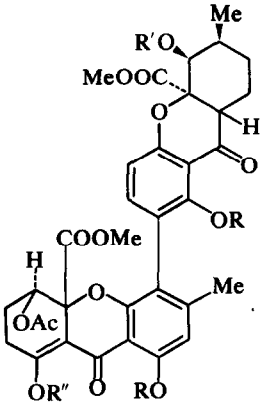
**2a** R=R′=R″=H
**2b** R=R′=R″=Ac
**2c** R=R″=H R′=Ac
**2d** R=R′=H R″=Me



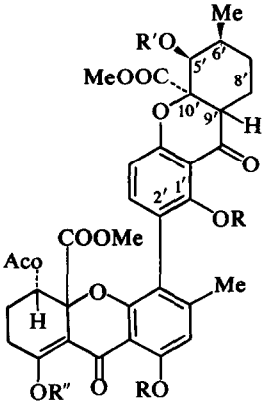
**3a′** R=R′=R″=H



**4a** R=R′=R″=H
**4b** R=R′=R″=Ac
**4c** R=R″=H R′=Ac
**4d** R=R′=H R″=Me



**3a′** R=R′=R″=H



**4a′** R=R′=R″=H

presence of an α-bromo- αβ-unsaturated 6-membered ring ketone (1706 $cm^{-1}$) and a hydrogen bonded aryl carbonyl (1672 $cm^{-1}$). In comparison with the NMR spectrum of tribromoeumitrin B, this compound revealed the appearance of an additional phenolic hydroxyl (δ 8·87) and the disappearance of a secondary alcoholic hydroxyl indicating that the monomeric moiety of tribromoeumitrin B having a secondary alcoholic group was drastically transformed on treatment with MeOH, while the other half bearing a secondary acetoxyl remained unchanged. The transformed compound gave well developed pale yellow plates when recrystallized repeatedly from MeOH and an X-ray analysis was undertaken.

The lattice constants and space group were

determined from several procession photographs, and the crystal density measured by the flotation method using KIaq. The crystal data observed are as follows: Ortho rhombic, space group $P_{2_12_12_1}$ $Z = 4$, $a = 12{\cdot}98$, $b = 33{\cdot}28$, $c = 8{\cdot}28$ Å; density measured: $1{\cdot}56\,g.cm^{-3}$; calc.: $1{\cdot}64\,g.cm^{-3}$. Three dimensional intensity data were recorded on the multiple-film equi-inclination Weissenberg photographs. The layer lines of zero to six around the *c*-axis and zero to two around the *b*-axis were taken with CuK $\alpha$ radiation.

The intensities were measured by a Narumi microdensitometer and corrected for Lorentz and polarization factors. No correction was applied to absorption.

The intensities of higher order reflexions decreased quite rapidly as the diffraction angle increases, which resulted in a rather large apparent overall temperature factor of about 10 Å.[2] Consequently, only 793 independent non-zero structure factors were obtained for the structure determination.

The crystal structure was determined by the heavy atom method and refined by the method of block-matrix least squares. In the later stage of the refinement, anisotropic temperature factors for all the atoms were allowed for. No hydrogen atom was included in the refinement. The oxygen atoms were identified on the basis of chemical and spectroscopical data. Although the final R value dropped as low as 0·09, the estimated standard deviations for most of the oxygen and carbon atoms were quite large (about 0·08 Å for the positional parameters, and 0·12 Å for the C—C and C—O bonds).

This was clearly caused by the large temperature factors and rather small number of observed structure factors as compared with the number of atoms.

The absolute configuration was determined by the use of anomalous dispersion of CuK$\alpha$ radiation by the bromine atoms. A comparison of the observed and calculated intensity ratios of the Friedel pairs of reflexions indicated the absolute configuration as given in Fig. 3 which also shows the stereostructure of the molecule.

As a monomeric half of tribromoeumitrin B suffered a drastic transformation on MeOH treatment, the X-ray structural determination of the final product (**6**) cannot provide any direct evidence for the absolute structure of that portion of the initial compound. The absolute structure of the total molecule of eumitrin B (**4a**) has finally been deduced from the reasonable elucidation of the rearrangement of tribromoeumitrin B (**5**) as illustrated in Chart 2 taking account of the relative configuration with respect to an angular carbomethoxyl, secondary alcohol and secondary methyl as given in the partial structure (**12**).

On the basis of the absolute structure of eumitrin B (**4a**), those of eumitrin $A_1$ (**2a**) and eumitrin $A_2$ (**3a**) have now been established.

## EXPERIMENTAL

M.p.'s determined on a Yanagimoto micro apparatus are uncorrected. UV spectra were measured on a Carry 11; ORD on a JASCO ORD/UV-5 (*J* 15) spectrometer and IR spectra on a JASCO DS-402G spectrophotometer; NMR spectra were taken with a JEOL JNM-PS-100 instrument (100 Hz) using TMS as internal reference. The mass spectra were recorded on a Hitachi (double focus) RMU-6E mass spectrometer and high resolution mass spectra on a JEOL JMS-OISG-2.

*Isolation and purification of eumitrins*

The crushed thalli of *Usnea bayleyi*(Stirt.)Zahlbr. subsp. *septentrionalis* collected at Yuriagehama near Sendai were continuously extracted with hot benzene for 2–3 days.

On concentration of the red coloured extracts, usnic acid separated and the filtrate was evaporated to dryness. The residue was treated with hot EtOH to remove insoluble precipitates, and the red ethanolic filtrate evaporated to dryness. The residue was treated with ether as above. Finally the yellow coloured hot hexane extract was chromatographed on silica gel impregnated with 0·5 N oxalic

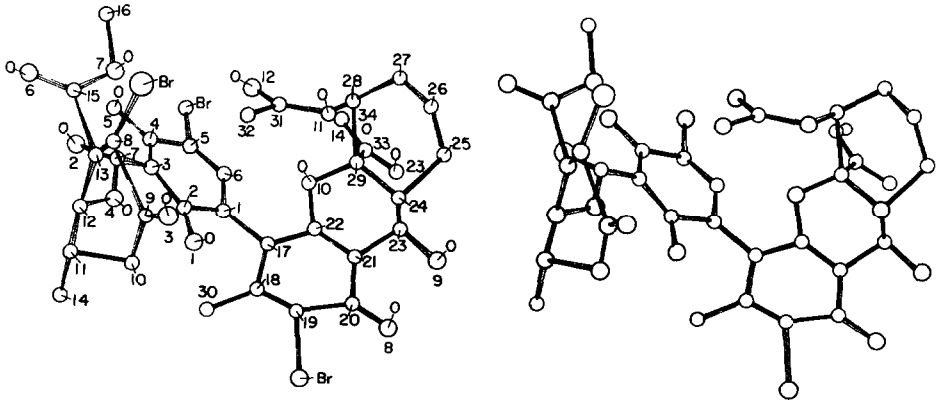


Fig 3. A stereoscopic view of the molecular structure of the transformed product of tribromoeumitrin B drawn by the plotter program *ORTEP*.

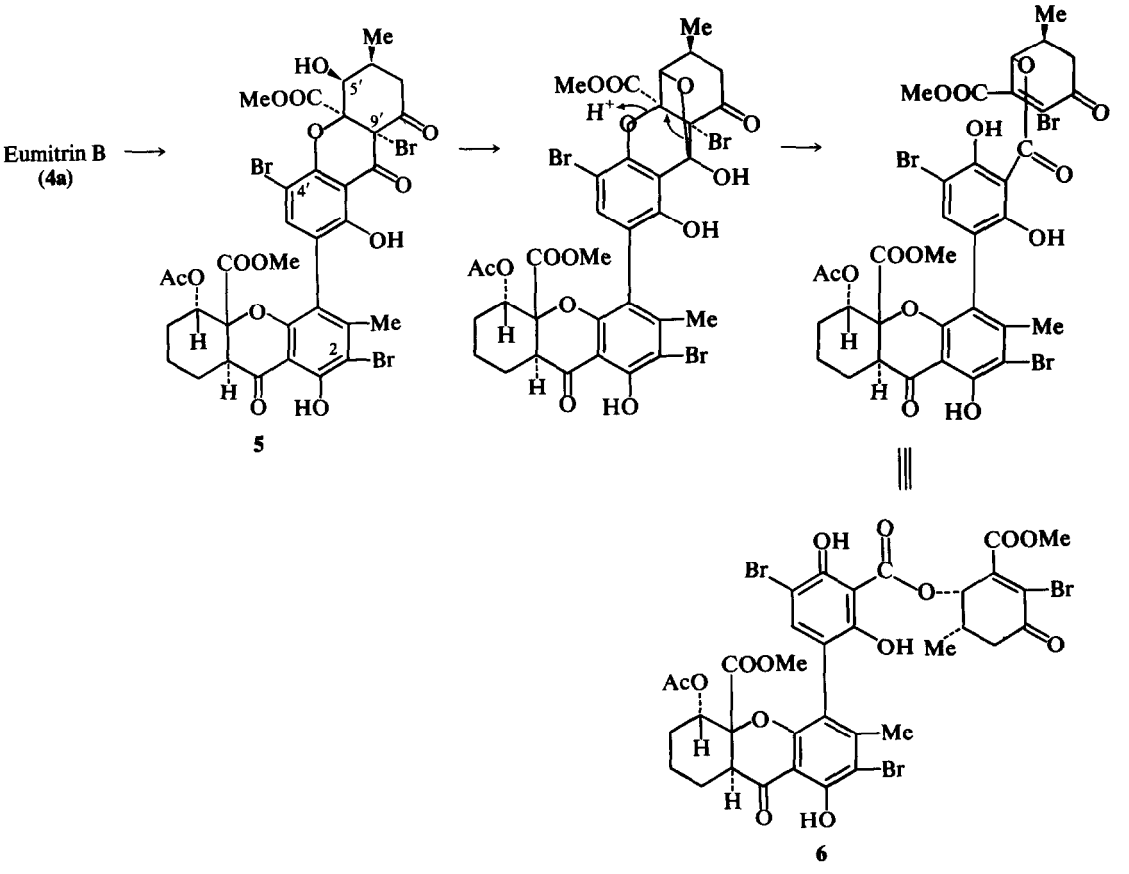



Chart 2.

acid to isolate (+)usnic acid, eumitrin B and eumitrin A mixture (elution with benzene then 2·5–3% (v/v) acetone in benzene).

Eumitrin B was recrystallized from EtOH-benzene (5:1) to give yellow prisms. The eumitrin A mixture was separated into $A_1$ and $A_2$ by careful recrystallization from a very small amount of hot EtOH-benzene mixture (5:1).

On allowing to stand at room temp., eumitrin $A_1$ crystallized out as yellow plates within 20 min which were separated by filtration. The filtrate was allowed to stand at room temp. without concentration, when eumitrin $A_2$ was obtained as pale yellow needles.

*Properties of eumitrins*

*Eumitrin* $A_1$ (**2a**). Yellow plates, m.p. 249–252° (from EtOH-benzene (5:1)), $[\alpha]_D$ −52·4° (in dioxan); $FeCl_3$: red brown; Gibbs' test: negative (Found: C, 60·56; H, 4·82. $C_{34}H_{32}O_{15}$ requires: C, 60·00; H, 4·74%). High resolution mass spectrum: mw 680·170 (Calc. 680·174).

*Eumitrin* $A_2$ (**3a**). Pale yellow needles, m.p. 216–220° (from EtOH-benzene (5:1)), $[\alpha]_D$ −76·7° (in dioxan), $FeCl_3$: greenish red brown; Gibbs' test: negative. (Found: C, 61·37, 5·19. $C_{34}H_{34}O_{14}$ requires: C, 61·26; H, 5·11%). High resolution mass spectrum mw 666·192 (Calc. 666·195).

*Eumitrin* B (**4a**). Yellow prisms, m.p. 238–240° (from EtOH-benzene (5:1)), $[\alpha]_D$ −33·3° (in dioxan); $FeCl_3$: greenish red brown; Gibbs' test: negative. (Found: C, 61·01; H, 5·34. $C_{34}H_{34}O_{14}$ requires: C, 61·26; H, 5·11%).

*Acetylation of eumitrins* $A_1$, $A_2$ *and* B *with acetic anhydride and pyridine*

The pigment was acetylated with $Ac_2O$ in pyridine by the usual way. The products were chromatographed on silica gel impregnated with 0·5 N oxalic acid eluting with benzene-acetone (9:1).

*Eumitrin* $A_1$ *peracetate* (**2b**). δ ($CDCl_3$): 1·03 (3H, d, *J* 6 Hz $\rangle$CH—C<u>H</u>$_3$), 1·90 (3H, s, —OCOC<u>H</u>$_3$), 2·06 (3H, s, Ar—C<u>H</u>$_3$), 2·01, 2·15, 2·22, 2·25, 2·35 (3H each, s, —OCOC<u>H</u>$_3$ × 5), 3·65, 3·75 (3H each, s, —COOC<u>H</u>$_3$ × 2), 5·32 (1H, m, $\rangle$C<u>H</u>—OAc), 5·65 (1H, br. s, $\rangle$C<u>H</u>—OAc), 6·54 (1H, s, Ar—<u>H</u>), 6·96, 7·31 (1H each AB q, *J* 8 Hz *ortho* coupling Ar—<u>H</u> × 2).

*Eumitrin* B *peracetate* (**4b**). δ ($CDCl_3$): 1·05 (3H, d, *J* 6 Hz, $\rangle$CH—C<u>H</u>$_3$), 1·95 (3H, s, —OCOC<u>H</u>$_3$), 2·07 (3H, s, Ar—C<u>H</u>$_3$), 2·01, 2·16, 2·23, 2·37 (3H each, s, —OCOC<u>H</u>$_3$ × 4), 3·65, 3·80 (3H each, s, —COOC<u>H</u>$_3$ × 2), 5·35 (1H, m, $\rangle$C<u>H</u>—OAc), 5·65 (1H, br. s, $\rangle$C<u>H</u>—OAc), 6·54 (1H, s, Ar—<u>H</u>), 6·96, 7·16 (1H each AB q, *J* 8 Hz *ortho* coupling Ar—<u>H</u> × 2).

*Acetylation of eumitrins* $A_1$ *and* B *with acetic anhydride and* p*-toluenesulphonic acid*

The pigment was acetylated with an excess of $Ac_2O$ and equivalent amount of *p*-TsOH . $H_2O$ with stirring for 1½ hr at room temp. The mixture was treated in the usual way to purify the products by chromatography on silica gel impregnated with 0·5N oxalic acid eluting with benzene-acetone (9:1).

*Eumitrin* $A_1$ *monoacetate* (**2c**). δ ($CDCl_3$): 1·03 (3H, d, *J* 6 Hz >CH—C<u>H</u>$_3$), 1·82 (3H, s, —OCOC<u>H</u>$_3$), 2·06 (3H, s, Ar—C<u>H</u>$_3$), 2·12 (3H, s, —OCOC<u>H</u>$_3$), 3·67, 3·74 (3H, each s, —COOC<u>H</u>$_3$ × 2), 5·30 (1H, m, >C<u>H</u>—OAc), 5·62 (1H, br.s, >C<u>H</u>—OAc), 6·43 (1H, s, Ar—H), 6·49, 7·15 (1H each, AB q, *J* 8 Hz *ortho* coupling Ar—H × 2), 11·19, 11·45 (1H each, s, phenolic OH × 2), 13·80, 13·89 (1H each s, enolic OH × 2).

*Eumitrin* B *monoacetate* (**4c**). δ ($CDCl_3$): 1·08 (3H, d *J* 6 Hz, >CH—C<u>H</u>$_3$), 1·90 (3H, s, —OCOC<u>H</u>$_3$), 2·09 (3H, s, Ar—C<u>H</u>$_3$), 2·15 (3H, s, —OCOC<u>H</u>$_3$), 3·69, 3·81 (3H each, s, —COOC<u>H</u>$_3$ × 2), 5·42 (1H, m, >C<u>H</u>—OAc). 5·67 (1H, br.s, >C<u>H</u>—OAc), 6·45 (1H, s Ar—H), 6·52, 7·05 (1H each, AB q *J* 8 Hz *ortho* coupling Ar—H × 2), 11·40, 11·45 (1H each, s, phenolic OH × 2), 13·87 (1H s, enolic OH).

*Methylation of eumitrins with diazomethane*

The pigment was methylated with a slight excess of $CH_2N_2$ in ether for 2–3 hr under ice cooling. The mixture was allowed to stand overnight at room temp. Excess $CH_2N_2$ was decomposed with a few drops of glacial AcOH. On treating as usual the product was purified by silicic acid column chromatography eluting with benzene-acetone (10:1 (v/v)).

*Eumitrin* $A_1$ *dimethyl ether* (**2d**). m.p. 146–150° δ ($CDCl_3$): 0·87 (3H, d *J* 6 Hz >CH—C<u>H</u>$_3$), 1·83 (3H, s, —OCOC<u>H</u>$_3$), 2·16 (3H, s, Ar—C<u>H</u>$_3$), 3·68, 3·76 (3H each s, —COOC<u>H</u>$_3$ × 2), 3·92, 3·97 (3H each s, OC<u>H</u>$_3$ × 2), 4·13 (1H br.s, >C<u>H</u>—OH), 5·32 (1H, m >C<u>H</u>—OAc), 6·42 (1H s, Ar—H), 6·52, 7·19 (1H each AB q, *J* 8 Hz *ortho* coupling Ar—H × 2), 12·50, 12·95 (1H each, s, phenolic OH × 2).

*Eumitrin* B *monomethyl ether* (**4d**). δ ($CDCl_3$): 0·9 (3H, d *J* 6 Hz >CH—C<u>H</u>$_3$), 1·90 (3H, s, —OCOC<u>H</u>$_3$), 2·01 (3H, s, Ar—C<u>H</u>$_3$), 3·32 (1H, m, >C—H), 3·69, 3·79 (3H, s, —COOC<u>H</u>$_3$), 3·97 (3H, s, —OC<u>H</u>$_3$), 4·17 (1H, br.s, >C<u>H</u>—OH), 5·45 (1H, m, >C<u>H</u>—OAc), 6·47 (1H, s, Ar—H), 6·54, 7·06 (1H each AB q, *J* 8 Hz, *ortho* coupling Ar—H × 2), 11·46, 13·03 (1H each phenolic OH × 2).

2,4′,9′-*Tribromoeumitrin* B (**5**). Yellow plates, m.p. 240°. Eumitrin B was added to a partly suspended mixture of $FeCl_3$ (80 mg) and $CHCl_3$ (20 ml), which became a red brown solution. To this solution was added dropwise an almost equivalent amount of bromine water under stirring and ice-cooling for 1½ hr. After 30 min at room temp, excess of bromine and $FeCl_3$ were destroyed with $Na_2S_2O_4$aq. The mixture was shaken with $CHCl_3$, and the lower layer water washed and dried. On removing solvent, the residue was chromatographed over silica gel impregnated with 0·5 N oxalic acid using benzene-acetone (20:1) as eluant. Dibromo derivative, m.p. 183°, was removed first, and tribromoeumitrin B was then obtained fairly pure.

$\nu_{max}^{KBr}$ $cm^{-1}$: 1754 (acetate C=O), 1745 (methyl ester C=O, and α-bromo six membered ring C=O), 1648 (H-bonded aryl C=O), 1613 (benzene ring): δ ($d_6$-acetone) 1·06 (3H, d, *J* 6 Hz, >CH—C<u>H</u>$_3$), 1·87 (3H, s, —OCOC<u>H</u>$_3$), 2·18 (3H, s, Ar—C<u>H</u>$_3$), 3·48 (1H, m, >C—H), 3·79, 3·89 (3H each, s, —COOC<u>H</u>$_3$ × 2), 4·46 (1H, m, >C<u>H</u>—OH bs, when $D_2O$ added), 5·39 (1H, m, >C<u>H</u>—OAc), 6·06 (1H, bs, >C<u>H</u>—OH disappeared when $D_2O$ added), 7·49 (1H, s, Ar—H), 10·58, 12·38 (1H each s, phenolic OH × 2). No enolic OH.

*The rearranged product from* 2,4′,9′*-tribromoeumitrin* B

2,4′,9′-Tribromoeumitrin B (**5**) was dissolved in MeOH. On cooling pale yellow plates, m.p. 224–225°, (**6**) separated. (Found: C, 45·09; H, 3·39; Br, 25·99. $C_{34}H_{31}O_{14}Br_3$ requires: C, 45·21; H, 3·46, Br, 26·54%). $\lambda_{max}^{Dioxan}$ nm (log ε): 264 (4·40), 362 (3·92). $\nu_{max}^{KBr}$ $cm^{-1}$: 1758 (acetate C=O), 1706 (α-bromo-αβ-unsaturated 6-membered ring C=O), 1672, 1657 (H-bonded aryl C=O), 1617 (benzene ring). δ (in $CDCl_3$): 1·26 (3H, d, *J* 5 Hz >C(C<u>H</u>$_3$)H), 1·48–1·86 (m, >C<u>H</u>$_2$ and >C—H), 1·95 (3H, each, s, —OCOOC<u>H</u>$_3$), 2·25 (3H, s, Ar—C<u>H</u>$_3$), 3·32 (1H, tr, *J* 10, 3 Hz, >C—H), 3·71, 3·92 (3H each, s, COOC<u>H</u>$_3$ × 2), 5·44 (1H, br s, $W_{1/2}$ 7·5 Hz, >C(OAc)H), 6·27 (1H, br.s, $W_{1/2}$ 5 Hz, >C(O—)H) 7·27 (1H, s, Ar—H), 8·87, 10·33, 12·30 (1H each, s, phenolic OH × 3).

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