# NEW AZASTEROIDAL ANTIFUNGAL ANTIBIOTICS FROM *GEOTRICHUM FLAVO-BRUNNEUM*

## II. ISOLATION AND CHARACTERIZATION

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A novel group of antibiotics, comprising microbiologically-active structurally-related factors A25822A, B, D, H, L, M and N, produced by culturing *Geotrichum flavo-brunneum* NRRL 3862 under submerged aerobic fermentation conditions was isolated by extraction. The individual factors were separated and purified by chromatography and crystallization. The major factor, A25822B, a 15-aza-24-methylene-D-homocholestadiene is a white crystalline compound, $C_{28}H_{45}NO$. The antibiotics are highly active against fungi and marginally active against bacteria.

A mixture of broad spectrum antifungal antibiotics was detected in the crude fermentation broth of the fungi *Geotrichum flavo-brunneum* NRRL 3862. The novel antibiotic complex was isolated by extraction of the whole fermentation broth or mycelium, and the structurally-related factors A25822A[1], B, D, H, L, M and N were separated and purified by chromatographic techniques and fractional crystallization. The complex, consisting of one major and six minor components, was highly active against a broad spectrum of pathogenic fungi, including *Candida* species and *Trichophyton* species.

A25822B, biologically the most active component, was inhibitory in a range of $<0.0312$ $\sim 5.0$ mcg/ml. The complex was also active against several gram-positive and gram-negative bacteria[3].

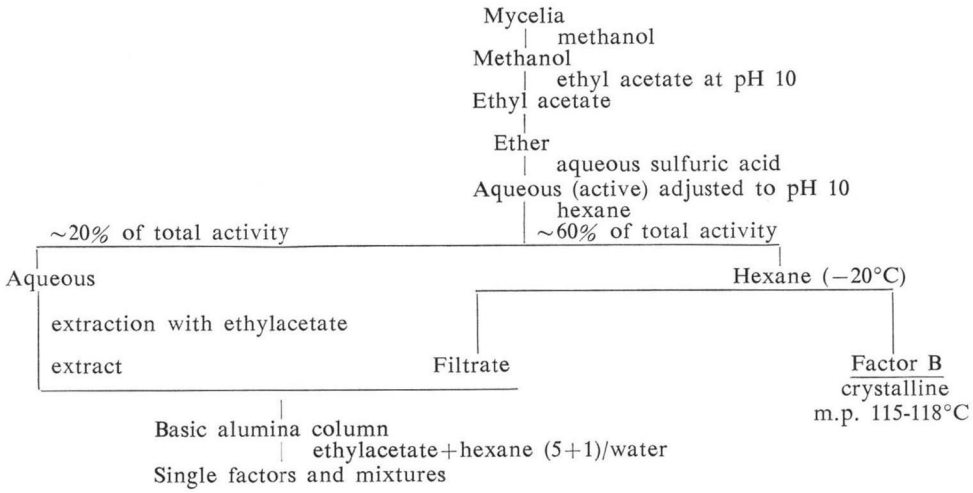
### Isolation

*Geotrichum flavo-brunneum* was grown under submerged aerobic fermentation conditions on a casein corn steep agar medium for 7 days. Approximately 10 % of the antibiotic synthesized was released into the fermentation broth while the remaining 90 % was retained by the cells[2].

The fermentation broth was filtered and the active principles extracted from the cells by multiple methanol extractions. The extracts were combined, concentrated to an aqueous phase and extracted with ethyl acetate at pH 10. The ethyl acetate extract was concentrated to an oily residue. The crude antibiotic complex was a brown, viscous oil which was soluble in most organic solvents, insoluble in water, and stable in solutions over a pH range of at least pH 3 to pH 10, at room temperature. The crude oil was dissolved in diethyl ether and the active compounds extracted with 0.2 N sulfuric acid solutions. The active components were re-extracted from the aqueous phase at pH 10 with *n*-hexane and ethyl acetate.

A25822B, the most abundant of the factors, was recovered from the *n*-hexane extract by

Fig. 1. Isolation for A-25822 factors A,B,D,H,L,M,N.




crystallization. The minor factors A, D, H, L, M and N were found in the ethyl acetate extract. The quantities of A25822 factors produced by a 100-liter fermentation are shown in Table 1.

The final purifications of factors A, D, H, L, M and N were achieved by chromatographic procedures and fractionated crystallization as described in detail in the experimental section of this paper.

Table 1. Ouantities of A-25822 produced by a 100-liter fermentation.

| Factor | Weight (g) |
|---|---|
| A | 1.00 |
| B | 5.00 |
| D | 0.20 |
| H | 0.05 |
| L | 0.10 |
| M | 0.10 |
| N | 0.05 |

## Physicochemical Properties

The characterization of the A25822 antibiotics is sufficiently complete to show the relationship among these individual compounds. Some physical and chemical properties are shown in Table 2.

Each factor is optically active and possesses a nitrogen atom as part of a chromophoric group, indicated by the shift of the ultraviolet maximum in acidic solutions. The pKa's, measured in 66 % DMF, range from 8.5 to 4.8 (see Table 2).

The molecular weights were determined by mass spectrometry, elemental analysis and electrometric titration in 66 % dimethylformamide (DMF). Each factor produced a strong molecular ion which in each case was identical with the base peak.

The elemental analysis of A25822H, an unstable and hygroscopic compound, did not correlate well with mass spectrometric data. However, extensive purification efforts, accompanied by repeated elemental analysis and high resolution mass spectrometric analysis led to the assignment of a tentative empirical formula of $C_{28}H_{43}NO_2$.

The A25822 antibiotics are soluble in ethanol, methanol, acetone, ethyl acetate, chloroform, *n*-butanol, *n*-butyl acetate and diethyl ether; slightly soluble in benzene, *n*-hexane,

Table 2. Physical and chemical properties of A-25822 active factors.

| Factor | Empirical formula | M.W. | M.P. °C | $[\alpha]_D^{25}$ | pKa | UV $\lambda$ max. | |
|---|---|---|---|---|---|---|---|
| | | | | | | (Base) | (Acid) |
| A | $C_{30}H_{49}NO$ | 439 | 147° | −72° | 8.5 | 239 nm | 278 nm |
| B | $C_{28}H_{45}NO$ | 411 | 115~118° | −16° | 8.5 | 239 nm | 278 nm |
| D | $C_{28}H_{45}NO_2$ | 427 | — | +39° | 7.7 | 235 nm | 270 nm |
| H | $C_{28}H_{43}NO_2$ | 425 | — | +15° | 6.9 | 235 nm | 270 nm |
| L | $C_{28}H_{43}NO_2$ | 425 | — | +75° | 4.8 | 262 nm | 278 nm |
| M | $C_{30}H_{47}NO_2$ | 453 | — | −15° | 7.9 | 239 nm | 278 nm |
| N | $C_{28}H_{43}NO$ | 409 | 165° | −14° | 7.5 | 239 nm | 278 nm |

Functional groups (UV, IR, NMR, MS, analyses) OH, C=C, basic N, C-$CH_3$.

*n*-pentane and petroleum ether; and insoluble in water. However, water-soluble salts can be prepared with acids. The individual factors give positive reactions with DRAGENDORFF's reagent and with 10 % phosphomolybdic acid in methanol on thin-layer plates.

## Discussion

Antibiotics A25822A and A25822B were assigned the structures 3A and 3B in Fig. 3 by CHAMBERLIN *et al.*[1]. We wish to report the structures of A25822M and A25822N as related to A25822B, as well as the relationship between A25822D and A25822L. No assignments could be made for A25822H.

As shown in Fig. 2, the ir spectra in chloroform of the A25822 factors are quite similar and exhibit absorption bands at ~3570 $cm^{-1}$ (OH), 1620 $cm^{-1}$ (conjugated double bond moiety) and 890 $cm^{-1}$ (exocyclic methylene). The ir spectra of A25822M exhibits a max 1730 $cm^{-1}$ indicating an ester carbonyl. Elemental analysis, mass spectrometric fragmentation and the appearance of a three-proton singlet at 2.0 ppm in the nmr spectra led to a suggestion that A25822M was identical with the $C_3$ acetyl derivative of A25822B ($R_3$ in Fig. 3 is $CH_3COO$-). For the nmr spectra of A25822M, see Fig. 4. These protons are missing in the nmr spectra of the other A25822 antibiotics. Hydrolysis of A25822M to A25822B with sodium methoxide confirmed this prediction.

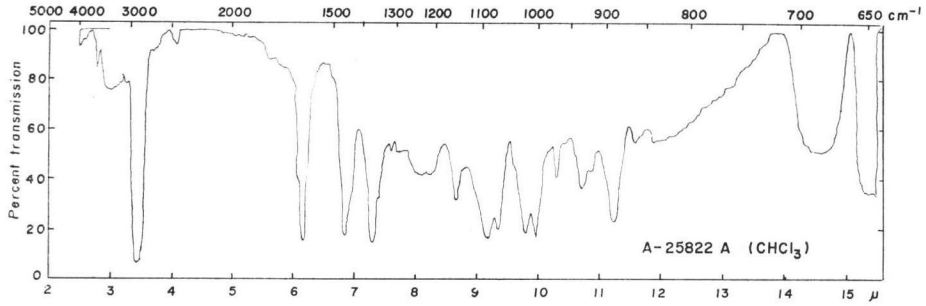
The ir spectra of A25822N exhibits a $\lambda$ max 1715 $cm^{-1}$, indicating a keto-carbonyl. On the basis of elemental analysis and mass spectrometric fragmentation, it was suggested that A25822N (MW 409) was closely related to A25822B (MW 411). Since the hydroxyl absorption in the ir spectra was missing, it was most logical that A25822N was the $C_3$-keto analog of A25822B. This was confirmed by the oxidation of A25822B to A25822N with JONES reagent in acetone. The structural relationship among the three antibiotics A25822B, M and N is, therefore, confirmed.

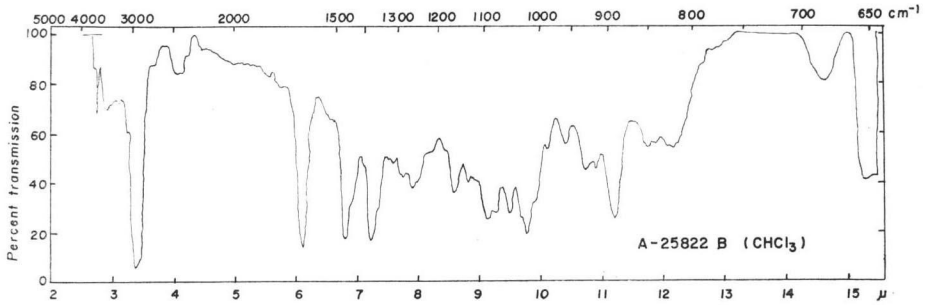
The antibiotics A25822M and A25822N are minor constituents of the complex. Both factors appear later in the fermentation than A25822B. It is suggested, therefore, that A25822B is converted by acetylation to A25822M and oxidation to A25822N during the fermentation process.

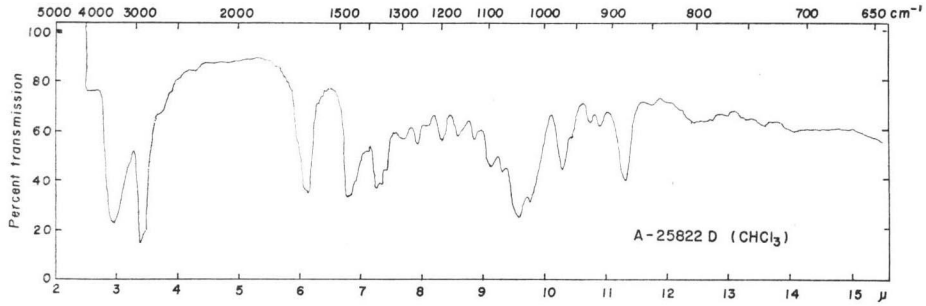
A25822D, A25822H and A25822L are minor constituents of the antibiotic mixture. They differ from A25822B mainly by their oxygen content.

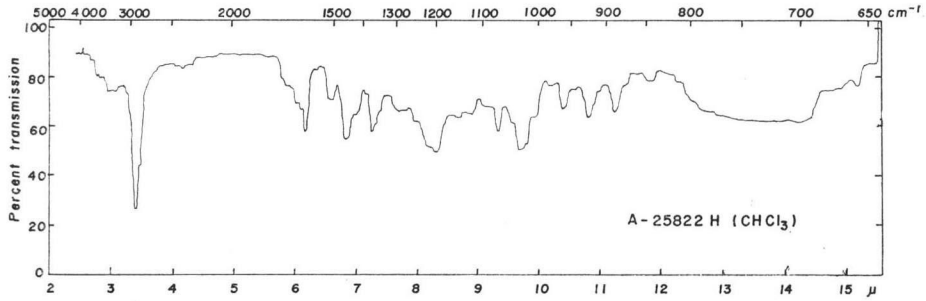
While complete chemical and physicochemical proof of their structures has not been possible because of insufficient quantities, the similarities in the infrared spectra, the mass spectrometric fragmentation patterns and biological properties justify a tentative assignment of general formulas for A25822D and A25822L as related to A25822B. The close relationship between these two antibiotics was also confirmed by preparation of their oxidation products.

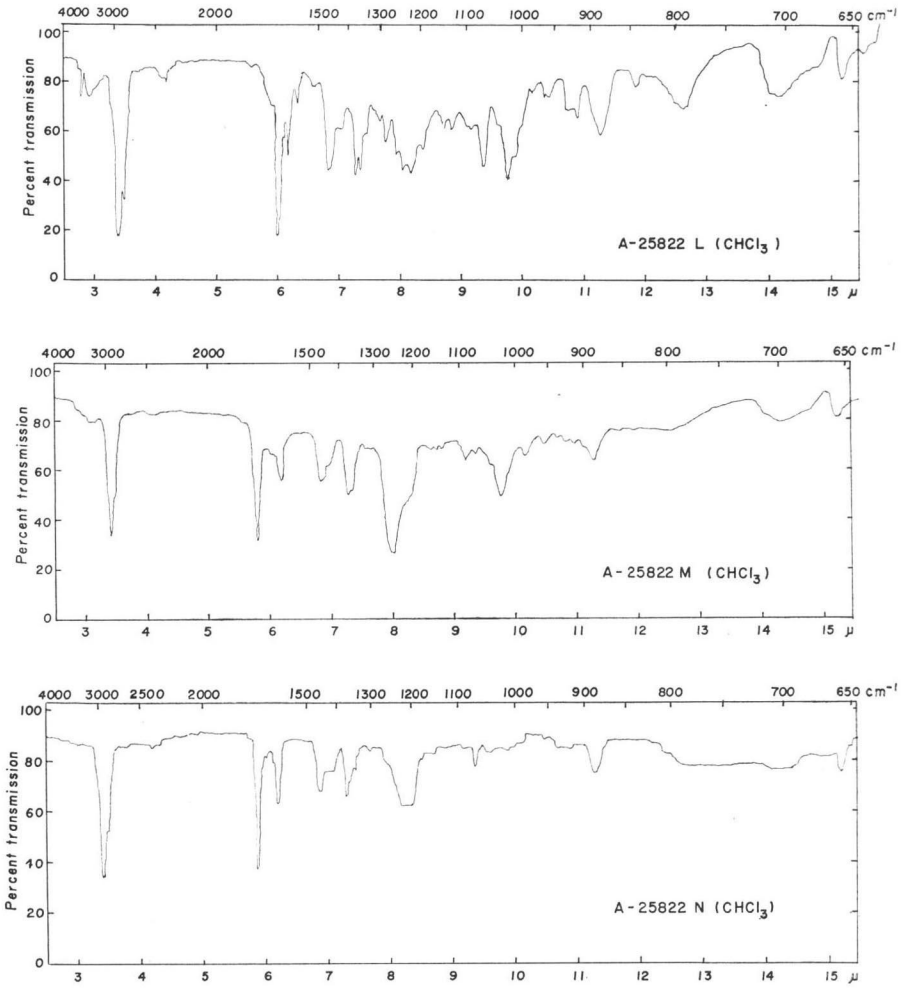
Fig. 2. IR spectra of A25822 antibiotics.



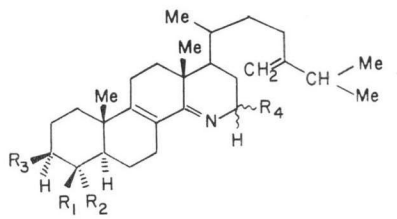



A25822D and A25822L oxidized with JONES reagent in acetone gave identical compounds (MW 423). The two oxidation products were identified by their uv, nmr, ir, ms and ord data.

One of the characteristics noticed after the oxidation of A25822D was the change in the uv absorption spectrum. A25822D showed a maxima at 235 nm and the oxidation product showed a maxima at 260 nm. This change could be explained by the introduction of a carbonyl in conjunction with an existing conjugated system (Fig. 3, $R_4$=O).

With the assumption that all seven A25822 antibiotics are biogenetically related to a 15-aza-D-homocholestadiene structure with a hydroxyl function at the $C_3$ position ($R_3$ in Fig. 3), compound A25822L would

Fig. 3. Structures of A25822 antibiotics A,B,M and N.




| | Factor | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|---|
| A 25822 | A* | $CH_3$ | $CH_3$ | OH | H |
| | B* | H | H | OH | H |
| | M | H | H | $-O-\overset{O}{\overset{\|}{C}}-CH_3$ | H |
| | N | H | H | =O | H |

* Structures A25822A and A25822B have been elucidated by J.W.CHAMBERLIN *et al.*[11]

have the structure of a conjugated ketoimine as shown in Fig. 3, Table 3. The absorptions at 1670~1685 $cm^{-1}$ in the ir spectra of A25822L and the pKa of 4.8 are in favor for this prediction.

Table 3. Tentative structures of A25822D and A25822L.

| Factor | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| A25822 D | H | H | OH | OH |
| L | H | H | OH | =O |

There is no preferable evidence that the keto function of A25822L is located at the $C_{11}$, however, this possibility cannot be ruled out completely. Since the oxidation products of A25822D and A25822L shown to be identical in all respects, A25822D has to be the hydroxyl analog of A25822L (Table 3, factor D). Some stereochemical aspects remain still to be determined.

## Experimental

### Isolation of Antibiotics A25822A, B, D, H, L, M and N

Extraction: A 900-liter fermentation of the mold *Geotrichum flavo-brunneum* was filtered, and the mycelium was stirred with 500 liters of methanol for 1 hour at room temperature. The methanol extract was concentrated to a 50-liter aqueous phase and adjusted to pH 10.0 with 5N sodium hydroxide. The basic solution was then extracted with an equal volume of ethyl acetate and the organic phase was concentrated to an oily residue.

The oil was dissolved in 20 liters of diethylether and gently mixed with an equal volume of 2.5 N aqueous sodium hydroxide solution. The aqueous phase was discarded, and the ether phase was washed three times with equal volumes of cold distilled water. The water washes were discarded, and the ether phase was extracted twice with half the volume of 0.2 N sulfuric acid. The organic phase was discarded.

The acidic aqueous extract was adjusted to pH 10.0 with sodium hydroxide solution and extracted with twice the volume of *n*-hexane (extract A) and twice the volume of ethyl acetate (extract B), respectively. Extract A, containing the bulk of the antibiotics A25822A, B, M, and N was concentrated under vacuum to a foam. Extract B was also concentrated under vacuum to a foam to give the more polar, minor components A25822D, H and L.

Crystallization of A25822B: Extract A (43 g) was dissolved in 50 ml of acetone and 150 ml of *n*-hexane. The clear solution was adjusted to one liter with *n*-hexane and kept at −20°C. Within 24 hours, 22 g of A25822B crystallized in small white needles, m.p. 115~118°C. The filtrate was combined with extract B(10 g) and concentrated to a foam under vacuum to give 32 g of extract C.

### Column Chromatographic Separation of Antibiotics A25822A, B, D, H, L, M and N

Extract C, containing additional quantities of A25822B and all the minor components, was dissolved in a minimum volume of the upper phase of an 80:16:4 mixture of ethyl acetate - *n*-hexane - water. The solution was applied to a 13-cm×63-cm column of basic alumina (Woelm grade W200, Water Associates, Inc.) packed in the upper phase of the same solvent combination. The column was eluted with the same solvent mixture. The identity of the different antibiotics in the column fractions was determined by TLC and GLC. For thin-layer chromatography the E. Merck standard silica gel plates were used in a solvent system of diethylether - ethanol (3:1). Gas-liquid chromatography

Table 4. Relative tlc Rf-values and glc retention times of A25822.

| Factor | Relative Rf-values (TLC) | Relative retention times (GLC) |
|---|---|---|
| A | 0.44 | 10.0 |
| B | 0.28 | 7.0 |
| D | 0.27 | 8.8 |
| H | 0.54 | 8.4 |
| L | 0.67 | 8.4 |
| M | 0.42 | 9.2 |
| N | 0.40 | 7.1 |
| Cholesterol (Standard) | | 4.0 |

was carried out on a four-foot glass column, 4 mm I.D. packed with 3% OV-101 on Chromosorb W (HP) 80/100 mesh, Pierce Chemical Co., Rockford; with a F&M Scientific, 402 High Efficiency Gas Chromatograph, Hewlett-Packard, Palo Alto, California; column temperature 275°C; flash heater temperature 285°C; hydrogen flow 47 ml/minute; helium flow 75 ml/minute; air flow 350 ml/minute. Relative thin-layer chromatographic Rf values and gas-liquid chromatographic retention times for the individual antibiotics are given in Table 4.

The column fractions containing the antibiotics were combined to yield the following combinations in partially purified form:

| Combination | I | II | III | IV | V |
|---|---|---|---|---|---|
| Factor | A25822M and N | A25822A and L | A25822B and H | A25822B | A25822D |

## Purification and Characterization of Antibiotics A25822A, B, D, H, L, M and N

Antibiotic A25822A: A solution of 6.0 g of combination II in 100 ml of acetone was left at 4°C for 24 hours to form a crystalline precipitate. The crystals were recrystallized from 50 ml of acetone at 4°C to yield 1.8 g of plates, m.p. 147°C (or needles, m.p. 133~136°C); $[\alpha]_D^{25}$ −72° (*c* 1.15, methanol); pKa 8.45 (66% DMF). Uv absorption: $\lambda_{max}$ (EtOH) 239 nm, neutral and basic pH (ε 12,600); $\lambda_{max}$ 278 nm, acidic pH (ε 14,600). Ir spectrum: ($CHCl_3$) 3584 $cm^{-1}$ (OH), 1621 $cm^{-1}$ (−C=C−C=N−), 890 $cm^{-1}$ (exocyclic methylene). For ir spectrum, see Fig. 2. The ord exhibited three maxima: $[\alpha]_{292}^{25}$ −2800°, $[\alpha]_{252}^{25}$ +6700°, and $[\alpha]_{220}^{25}$ −1400° (*c* 0.070, methanol).

Analysis: Calculated for $C_{30}H_{49}NO$ ($M^+$ 439): C, 82.00; H, 11.25; N, 3.19; O, 3.56.
Found: C, 82.17; H, 11.31; N, 3.16; O, 3.40.

Antibiotic A25822B: The crystallization of A25822B, as described above, gave a white compound with a melting point of 115~118°C. Polymorphic forms with melting points of 42~44°C, 56~58°C or 88~90°C were obtained when other solvents and solvent combinations were used for crystallization of the antibiotic at different temperatures. These polymorphic crystals gave different X-ray powder pattern, but identical spectral and elemental analysis data in all respects: $[\alpha]_D^{25}$ −16° (*c* 0.775, methanol); pKa 8.5 (66% DMF). Uv absorption: $\lambda_{max}$ (EtOH) 239 nm, neutral and basic pH (ε 12,300); $\lambda_{max}$ (EtOH) 278 nm, acidic pH (ε 12,400). The ir spectrum: ($CHCl_3$) 3571 $cm^{-1}$ (OH), 1618 $cm^{-1}$ (−C=C−C=N−), 890 $cm^{-1}$ (exocyclic methylene). For ir spectrum, see Fig. 2. The ord exhibited three maxima: $[\alpha]_{294}^{25}$ −3100°, $[\alpha]_{252}^{25}$ +7100°, and $[\alpha]_{220}^{25}$ −2100° (*c* 0.096, methanol).

Analysis: Calculated for $C_{28}H_{45}NO$ ($M^+$ 411): C, 81.75; H, 10.95; N, 3.41; O, 3.89.
Found: C, 81.76; H, 10.71; N, 3.36; O, 3.99.

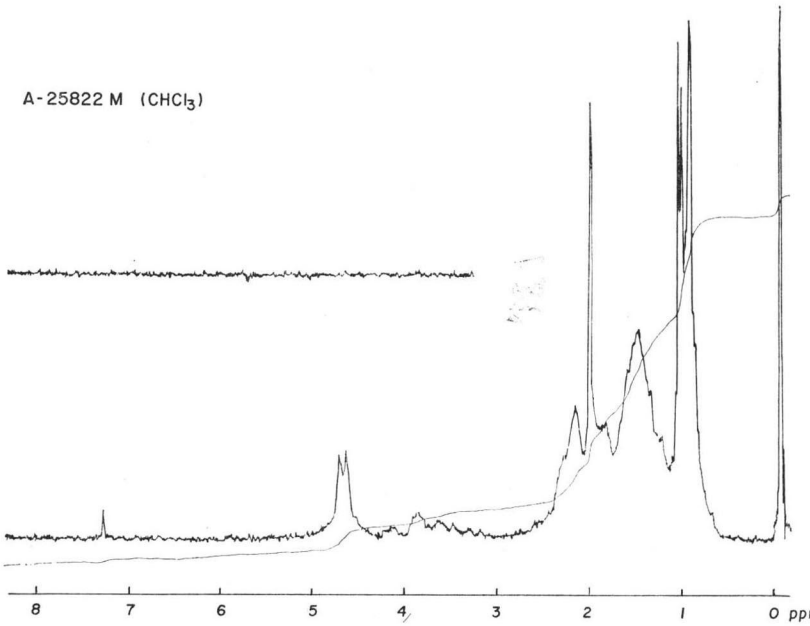
Antibiotic A25822D: The chloroform solution of 2.5 g of combination V was chromatographed on a 4.6 cm×56 cm column of grade 62 silica gel. The column was eluted with chloroform to which was added a linear methanol gradient of 0~40%. Twenty-ml fractions were collected. A25822D was eluted in fractions 135~170 which were combined and concentrated under vacuum to a dry foam, yielding 0.81 g of an amorphous material. This antibiotic could not be crystallized.

$[\alpha]_D^{25}$ +39° (*c* 0.722, methanol); pKa 7.7 (66% DMF). Uv absorption: $\lambda_{max}$ (EtOH) 235 nm, neutral and basic pH (ε 10,400); $\lambda_{max}$ (EtOH) 270 nm, acidic pH (ε 8,600). The ir spectrum exhibited absorption bands at 3600 $cm^{-1}$(OH), 1640 $cm^{-1}$ (−C=C−, −C=N−) and 890 $cm^{-1}$ (exocyclic methylene); thus, similar to antibiotics A25822A and A25822B. For ir spectrum, see Fig. 2.

Analysis: Calculated for $C_{28}H_{45}NO_2$ ($M^+$ 427): C, 78.69; H, 10.54; N, 3.28; O, 7.49.
Found: C, 78.40; H, 10.28; N, 3.17; O, 8.06.

A25822D (50 mg) in 2 ml of acetone was oxidized with 0.1 ml of JONES reagent (2.6 mmole of $CrO_3$ in acetone) and gave a dioxo-compound ($M^+$ 423); $[\alpha]_D^{25}$ +122° (*c* 0.015, methanol). Uv absorption: $\lambda_{max}$ (EtOH) 262 nm, neutral and basic pH (ε 9800) and $\lambda_{max}$ (EtOH) 278 nm, acidic pH (ε 9200). This compound was identical in all respects with an oxidation product

Fig. 4. NMR spectra of A25822M.




of A25822L. The ir showed ($CHCl_3$) 1715 $cm^{-1}$ (carbonyl); 1672 $cm^{-1}$ (amide) and 890 $cm^{-1}$ (exocyclic methylene). Hydroxyl bands were absent. The shift from 235 nm for A25822D to 262 nm for the oxidation product in the ultraviolet spectrum could be explained by the introduction of a carbonyl in conjugation to a previously existing conjugated system. The ord showed two positive maxima: $[\alpha]_{205}^{25}+18000°$, $[\alpha]_{272}^{25}+7500°$; and two negative maxima: $[\alpha]_{235}^{25}-4500°$, $[\alpha]_{330}^{25}-170°$ (*c* 0.026 in methanol). The compound crystallized from pentane, m.p. ~120°C; molecular ion and base peak 423.

Antibiotic A25822H: A chloroform solution of 8.0 g of combination III was chromatographed on a 6.2 cm×12 cm column of deactivated (6.5 % water) Woelm basic alumina. The column was then eluted with chloroform in 20-ml fractions. A25822H was eluted in fractions 81~210. These fractions were combined and concentrated under vacuum to an oil which was dissolved in 4 ml of acetone. The acetone solution was applied equally to six separate preparative thin-layer chromatography plates (E. Merck, 20 cm×20 cm pre-coated, silica gel F-254, layer thickness 2.0 mm on glass, Brinkmann Instruments, Inc.), which were developed in 3:1 diethyl ether-ethanol. The area on each plate containing A25822H was scraped off and combined. The silica gel was air-dried and applied to the top of a 6.2 cm ×30 cm column of silica gel (grade 62, 60~200 mesh, Matheson, Coleman & Bell) in ethyl acetate. The column was eluted with one liter of ethyl acetate which was concentrated under vacuum to a dry foam, yielding 0.146 g of white amorphous A25822H: $[\alpha]_D^{25}+15°$ (*c* 0.147, methanol); pKa 6.9 (66 % DMF). Uv absorption: $\lambda_{max}$ (EtOH) 235 nm, neutral and basic pH ($\varepsilon$ 10,000), $\lambda_{max}$ (EtOH) 270 nm, acidic pH ($\varepsilon$ 8,700). The ir spectra showed: ($CHCl_3$) 3550 $cm^{-1}$ (OH), 1625 $cm^{-1}$ (—C=C—, —C=N—), and 890 $cm^{-1}$ (exocyclic methylene). For ir spectrum, see Fig. 2.

Analysis: Calculated for $C_{28}H_{43}NO_2$ ($M^+$ 425)+$H_2O$ (MW 443):
C, 75.84; H, 10.20; N, 3.16; O, 10.80.
Found: C, 74.50; H, 10.15; N, 3.54; O, 11.36.

A25822H was found to be isomeric with A25822L. The ultraviolet absorption maxima and the pKa values were different. Both compounds could also be separated by TLC. Both antibiotics have the same empirical formula as determined by elemental analysis and high

resolution mass spectrometry. However, the elemental analysis data indicated that A25822H possesses strong hygroscopic properties, and the empirical working formula $C_{28}H_{43}NO_2(H_2O)$ was accepted as a realistic approach.

Antibiotic A25822L: Six grams of combination II were treated as described under A25822A. The filtrate from the A25822A crystallization was concentrated under vaccum to an oily residue and dissolved in a minimum volume of chloroform. The solution was applied to the top of a 1.7 cm×58 cm column of dry silica gel (Woelm dry column grade, activity III/30 mm) and eluted with chloroform. After the elution of 2.3 column volumes of chloroform, A25822L was collected in one column volume of effluent which was concentrated under vacuum to a dry foam, yielding 0.19 g of an amorphous powder: $[\alpha]_D^{25}+75°$ (*c* 0.072, methanol); pKa 4.75 (66 % DMF). Uv absorption: $\lambda_{max}$ (EtOH) 262 nm, neutral and basic pH (ε 10,200), $\lambda_{max}$ (EtOH) 278 nm, acidic pH (ε 10,800). The ord exhibited four maxima: $[\alpha]_{360}^{25}$ −270°, $[\alpha]_{272}^{25}$ +7550°, $[\alpha]_{235}^{25}$ −3500°, and $[\alpha]_{206}^{25}$ +16,700° (*c* 0.072, methanol). The ir spectra showed: ($CHCl_3$); 3620 $cm^{-1}$ (OH), 1670~1685 $cm^{-1}$ (amide), and 890 $cm^{-1}$ (exocyclic methylene). For ir spectrum, see Fig. 2.

Analysis: Calculated for $C_{28}H_{43}NO_2$ ($M^+$ 425): C, 79.01; H, 10.18; N, 3.29; O, 7.52.
Found: C, 78.66; H, 9.74; N, 3.06; O, 8.37.

Oxidation of 50 mg of A25822L in 2 ml of acetone with 0.1 ml of JONES reagent (2.6 mmole $CrO_3$ in acetone) gave an oxocompound ($M^+$ 423). This product gave identical uv, ord and ir spectral data and an identical mass spectrometric fragmentation pattern as the oxidation product from A25822D: $[\alpha]_D^{25}$ +110° (*c* 0.014, methanol); uv $\lambda_{max}$ (EtOH) 262 nm, neutral and basic pH (ε 9,900); $\lambda_{max}$ (EtOH) 278 nm, acidic pH (ε 9,300); ir ($CHCl_3$) 1715 $cm^{-1}$ (carbonyl), 1672 $cm^{-1}$ (amide).

Antibiotic A25822M: The filtrate from the A25822N crystallization was concentrated under vacuum to an oily residue which was dissolved in a minimum amount of chloroform. This solution was chromatographed on a 1.0 cm×25 cm column of deactivated (6 % water) Woelm basic alumina. The column was eluted in 10-ml fractions with chloroform. Fractions 18~31, containing A25822M, were combined and concentrated to an oily residue which was dissolved in benzene. The benzene solution was lyophilized to yield 0.334 g of a yellowish amorphous powder. A25822M could not be crystallized: $[\alpha]_D^{25}$ −15° (*c* 0.021, methanol); pKa 7.9 (66 % DMF). Uv absorption: $\lambda_{max}$ (EtOH) 239 nm, neutral and basic pH (ε 13,900), $\lambda_{max}$ (EtOH) 278 nm, acidic pH (ε 14,200). The ir spectra showed: 1730 $cm^{-1}$ (carbonyl) 1625 $cm^{-1}$ (−C−C=C−, −C=N−), 890 $cm^{-1}$ (exocyclic methylene). For ir spectra, see Fig. 2.

Analysis: Calculated for $C_{30}H_{47}NO_2$ ($M^+$ 453): C, 79.42; H, 10.44; N, 3.09; O, 7.05.
Found: C, 79.57; H, 10.26; N, 2.92; O, 7.21.

Mild treatment of the compound with sodium methoxide in methonol gave a product in 80 % yields identical in all aspects with A25822B. A25822B was then acetylated with acetic anhydride in pyridine to yield a product identical in all respects with A25822M.

Antibiotic A25822N: A solution of 0.5 g of combination I in 20 ml of acetone was placed at −20°C to form 0.039 g of a white crystalline precipitate of A25822N, melting point 165°C: $[\alpha]_D^{25}$ −14° (*c* 0.05, methanol), pKa 7.45 (66 % DMF). Uv absorption: $\lambda_{max}$ (EtOH) 239 nm, neutral and basic pH (ε 13,900), $\lambda_{max}$ (EtOH) 278 nm, acidic pH (ε 14,100). The ir spectra showed: 1715 $cm^{-1}$ (carbonyl); 1620 $cm^{-1}$ (−C=C−, −C=N−); 890 $cm^{-1}$ (exocyclic methylene). For ir spectra, see Figure 2.

Analysis: Calculated for $C_{28}H_{43}NO$ ($M^+$ 409): C, 82.16; H, 10.52; N, 3.43; O, 3.89.
Found: C, 81.82; H, 10.61; N, 3.53; O, 3.91.

Oxidation of A25822B with JONES reagent, in acetone as described under A25822L gave a reaction product in 80 % yields identical in all respects with A25522N. The structural relationship between these two antibiotics was, therefore, established.

Other A25822 Metabolites: The metabolites A25822C, E, F, G, J, and K have been

purified at various stages of the isolation process but have shown little or no biological activity. A25822J was identified as ergosterol ($C_{28}H_{44}O$) by physical-chemical methods. The relative retention time of ergosterol in our glc system (Table 4) was found to be 4.7. The other metabolites have not been fully characterized.

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## References

1) CHAMBERLIN, J.W.; M.O. CHANEY, S. CHEN, P.V. DEMARCO, N.D. JONES & J.L. OCCOLOWITZ: Structure of antibiotic A25822B, a novel nitrogen-containing $C_{28}$-sterol with antifungal properties. J. Antibiotics 27: 992~993, 1974
2) BOECK, L.D.; M.M. HOEHN, J.E. WESTHEAD, R.M. WOLTER & D.N. THOMAS: New azasteroidal antifungal antibiotics from *Geotrichum flavo-brunneum*. I. Discovery and fermentation. J. Antibiotics 28: 95~101, 1975
3) GORDEE, R.S. & T.F. BUTLER: New azasteroidal antifungal antibiotics from *Geotrichum flavo-brunneum*. III. Biological activity. J. Antibiotics 28: 112~117, 1975