MICROBIAL PRODUCTS. VI[1]

FIVE NOVEL METABOLITES RELATED TO BENZ[A]ANTHRACENE FROM AN UNIDENTIFIED ACTINOMYCETE DESIGNATED X-14881

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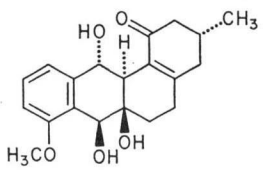
Five metabolites of actinomycete X-14881 were isolated from the fermentation broth and characterized. The major component was identified as [3*R*-(3α,6aβ,7β,12α,12aα)] or [3*S*-(3β, 6aα, 7α,12β,12aβ)]-6a, 7,12-trihydroxy-3, 4, 6a, 7, 12,12a-hexahydro-8-methoxy-3-methylbenz[a]-anthracen-1(2*H*)-one; the other four are closely related derivatives thereof.

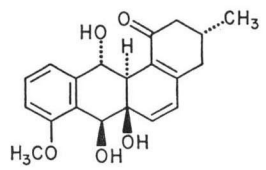
Actinomycete X-14881 produces an antibiotic complex, shown to be inactive *in vivo*, together with a number of other secondary metabolites, five of which were isolated and identified. All five possess the carbon skeleton of benz[a]anthracene. These compounds were designated X-14881 A~E (**1~5**) and showed no significant antimicrobial activity *in vitro*. X-14881 B was produced in larger quantities than any of the other four and did not exhibit any interesting pharmacological effects.

In this paper we describe the fermentation, isolation and chemical characterization of X-14881 A~E. The constitution and relative configuration of component X-14881 A (**1**) could be ascertained by single-crystal Roentgen-diffraction analysis. The extent of the chemical characterization of the other components depended on the quantities of material available Stereochemical aspects shown in **2~4** were extrapolated from X-14881 A.

The graphic representations of **1~4** contain stereodescriptors specifically denoting relative configuration of enantiomerically pure compounds according to a recently proposed convention.[2]



1 X-14881 A



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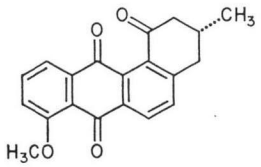
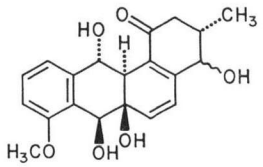
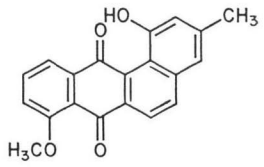


3 X-14881 C



4 X-14881 D



5 X-14881 E

Fermentations with Actinomycete X-14881

The actinomycete strain X-14881 was isolated from a soil sample collected in Lovell, Wyoming, U.S.A. and was cultivated and maintained in a starch-casein agar slant, composed of soluble starch 1.0, casein 0.1, dipotassium hydrogen phosphate 0.05, magnesium sulfate 0.05 and agar 1.5%, with pH adjustment to 7.4. A first-stage inoculum fermentation was prepared in a 500-ml Erlenmeyer flask containing Amidex starch (Corn Products Co. International, Englewood Cliffs, N.J.) 50 g/liter, Cerelose (technical grade glucose, Corn Products Co. International) 5 g/liter, Nutrisoy grit (Archer Daniels Midland Co., Decatur, Ill.) 35 g/liter, calcium carbonate 7 g/liter, and cobalt(II) chloride hexahydrate 0.24 mg/liter, with pH adjustment to 7.0 prior to sterilization. Spores and mycelium from an agar slant were used, and cultivation for 3 days on a rotary shaker at 250 rpm at 28°C furnished a broth, 3% (v/v) of which was used for a second-stage inoculum in 6-liter Erlenmeyer flasks containing 2 liters per flask of the medium described above. The resulting broth (4 liters), obtained after 72 hours under the same culture conditions, served as inoculum of a fermentation with 240 liters of the same medium. This fermentation was maintained for 4 days at 28°C with an aeration rate of 0.085 m^3/minute and an impeller speed of 280 rpm.

Isolation of Metabolites

The whole broth was adjusted to pH 6.0 (phosphoric acid) and filtered. The bulk of the antibiotic activity, as judged by agar-diffusion assays with *Staphylococcus aureus* ATCC 6538P as test organism, could be extracted from the broth filtrate at pH 10 with two ethyl acetate extractions (120 liters each). The combined ethyl acetate phases were washed with water (25 liters) and concentrated. The resulting syrup was distributed in three separatory funnels by the method of complete withdrawal[8] employing a mixture of acetonitrile and hexane (500 ml per phase). After the completed distribution, the acetonitrile phase contained in funnel No. 0 (r-series) was evaporated, the residue taken up in methanol and crude **1** (X-14881 A) deposited upon refrigeration. Two recrystallizations from chloroform - methanol (*ca.* 4: 1) gave pure **1** (4.4 g).

The mother liquor of the first crystallization was evaporated, and a portion (10 g) of the residue (54 g) was chromatographed on a column of Sephadex LH-20 (5×47 cm) with methanol as mobile phase. The first cut (500~620 ml) gave a crystalline residue on evaporation which was recrystallized three times from acetone-methanol-water and three times from acetone-methanol yielding crude **2** (65 mg) subsequently purified by preparative TLC (system 1) and crystallized from acetone-methanol-water.

The second cut (621~760 ml) was evaporated and a portion of the residue was chromatographed on a column of silica gel (Lobar, LiChroprep Si 60, E. Merck, Darmstadt) first with dichloromethane, and later with a linear dichloromethane - acetone gradient, as mobile phase. Evaporation of the fractions gave crystalline residues containing, in the order of progressing elution, components **5**, **3**, **2**, **1**, and **4**. Final purification of each component was achieved by preparative TLC with system 2 with the exception of **5** which was chromatographed in dichloromethane.

Materials, Apparatus and Methods

Amidex starch and Cerelose were purchased from Corn Products Co. International, Englewood Cliffs, N. J., U.S.A. and Nutrisoy grit from Archer Daniels Midland Co., Decatur, Ill., U.S.A. TLC was performed on precoated silica gel 60 F-254 plates, 0.25 mm thickness; for preparative work plates with layer thickness of 2 mm were employed (E. Merck, Darmstadt). The following solvent systems

(v/v) were used: dichloromethane - methanol (8: 1, system 1) and dichloromethane - acetone (9: 1, system 2). Melting points were determined without corrections on a Thermopan hot stage (Reichert), UV (Cary 14), IR (Digilab FTS-14), and ^{1}H NMR spectra (Varian XL-100) were recorded with the indicated solvents, and mass spectra were obtained with a Varian MAT CH5 instrument (70 eV, 250°C ion source temperature). Roentgen intensity data of **1** were measured on a Hilger-Watts diffractometer by $\theta-2\theta$ scans with Ni filtered Cu K_α radiation and pulse height discrimination. The crystal used for data collection measured approximately $0.15\times0.20\times0.6$ mm; the data were not corrected for absorption.

Structure Determinations

[3*R*-(3α, 6aβ, 7β, 12α, 12aα)] or [3*S*-(3β, 6aα, 7α, 12β, 12aβ)]-6a,7,12-Trihydroxy-3,4,5,6,6a,7,12,12a-octahydro-8-methoxy-3-methylbenz[a]anthracen-1(2*H*)-one (**1**, X-14881 A)

Component X-14881 A was isolated first and obtained as white prisms, mp>215°C (dec), $[\alpha]_D^{25}+379.1°$ (*c* 0.8, chloroform), Rf 0.44 (system 2), UV max (chloroform) 280 (ε 3,500) and 248 nm (10,300), IR (KBr) 1624 cm^{-1} (hydrogen-bonded, conjugated carbonyl) (see Fig. 1), ^{1}H NMR ($CDCl_3$) δ 1.05 (d, CH_3C, J=5.5 Hz), 1.05~1.55 (m, 2, H6), 1.8~3.0 (m, 8, H2-H5, H12a), 2.54 (s, 1, OH), 3.88 (s, CH_3O), 4.65 (s, 1, OH), 4.75 (s, 1, H7), 4.85 (dd, 1, H12, $J_{12,OH}$=4.5 and $J_{12,12a}$=9.5 Hz), 5.70 (d, 1, C12-OH, J=4.5 Hz), 6.81 (dd, 1, H9, $J_{9,10}$=7.5 and $J_{9,11}$=2.5 Hz), and 7.33 (m, 2, H10 and H11), mass spectrum, *m/z* (relative intensity) 326 (19, M$-H_2O$), 309 (28), 308 (100, M$-2H_2O$), 293 (13), 291 (7, M$-3H_2O$).

Anal. Calcd. for $C_{20}H_{24}O_5$: C 69.75, H 7.02.
Found: C 69.58, H 7.02.

The assigned structure of **1** is based primarily on a Roentgen-diffraction analysis of a crystal with space group $P2_12_12_1$, a=6.812(1), b=14.401(2), c=17.815(2) Å, and d (calcd.)=1.308 gcm^{-3} for Z=4. Of the 1391 independent reflections with $\theta<57°$, 1324 were considered to be above background [I>2.5 σ(I)]. The structure was solved by a multiple solution procedure[4] and was refined by full matrix least squares. In the final refinement, anisotropic thermal parameters were used for the heavier atoms and isotropic temperature factors were used for the hydrogen atoms. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indices are

Fig. 1. IR spectra of X-14881 A~D.

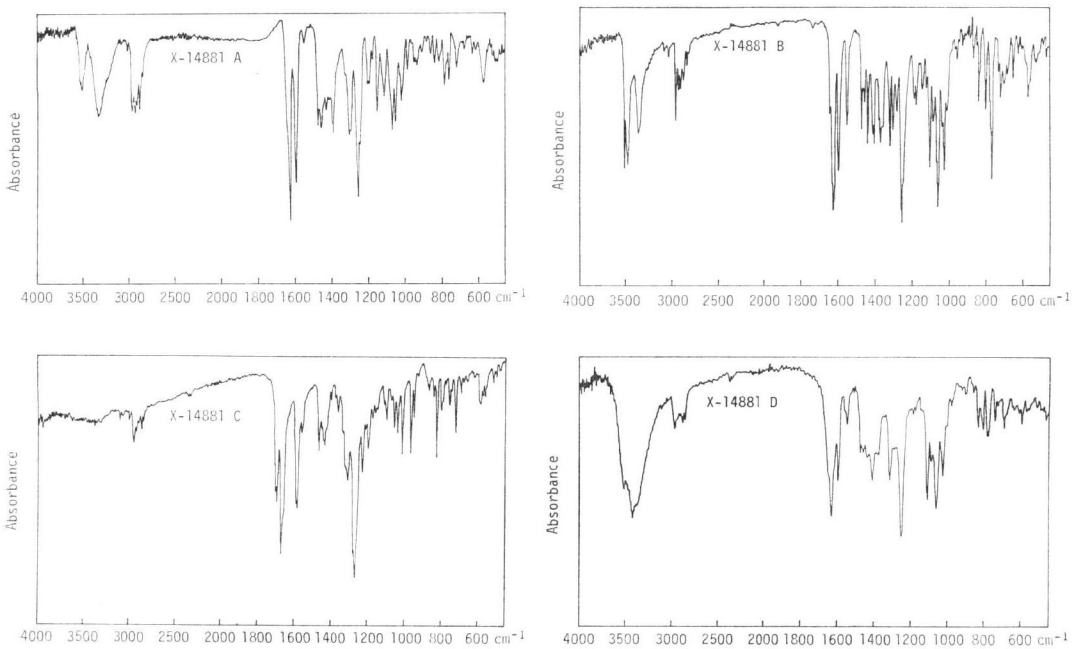
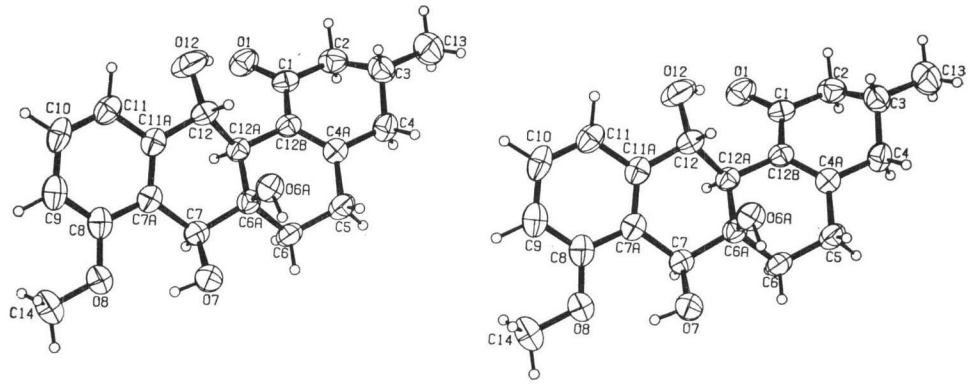


Fig. 2. A stereoscopic drawing of a molecule of **1** showing its conformation and relative stereochemistry.



R=0.033 and wR=0.43 for the 1324 observed reflections. The final difference map has no peaks greater than ± 0.2 eÅ^{-3}. The final atomic parameters and the anisotropic thermal parameters have been deposited with the Crystallographic Data Centre. A stereoscopic view of **1** is shown in Fig. 2.

[3*R*-(3α,6aβ,7β,12α,12aα)] or [3*S*-(3β,6aα,7α,12β,12aβ)]-6a,7,12-Trihydroxy-3,4,6a,7,12,12a-hexahydro-8-methoxy-3-methylbenz[a]anthracen-1(2*H*)-one (**2**, X-14881 B)

Produced in the fermentation as the major component, **2** appeared chromatographically very closely related to **1**. The UV maximum at 248 nm which was observed in **1** and attributed to an α,β-conjugated ketone was replaced in the spectrum of **2** by a maximum at 300 nm suggesting the presence of an additional conjugated double bond to form a dienone. This added unsaturation appeared to be the only feature differentiating **2** from **1**; although the molecular ion in the mass spectrum of **2** was less than 1%, it could be observed at *m/z* 342 and was strongly supported by two peaks corresponding to two successive dehydrations so that the molecular formula of $C_{20}H_{24}O_5$ was likely. The ^{1}H NMR spectrum conspicuously revealed the Me-C*H* proton as a doublet of identical shape observed in **1** so that the double-bond location at C3-C4 was excluded. A new *cis*-olefinic AB-pattern established the correct location and hence the structure **2**.

X-14881 B was obtained as off-white crystals, mp>218°C (dec), $[\alpha]_D^{25}$ +233.6° (*c* 0.4, chloroform), Rf 0.50 (system 2), UV max (chloroform) 300 (ε 8,860) and 282 nm (7,370), IR (KBr) 1625 cm^{-1} (hydrogen-bonded, conjugated carbonyl, Fig. 1), ^{1}H NMR ($CDCl_3$) δ 1.08 (d, CH_3C, J=5.5 Hz), 2.05~2.90 (m, 6, H2-H4, H12a), 2.77 (s, 1, OH), 3.88 (s, CH_3O), 4.95 (s, 1, H7), 5.77 (s, 1, OH), 5.32 (dd, 1, H12, $J_{12,OH}$=3.5 and $J_{12,12a}$=8.5 Hz), 6.17, 6.78 (AB, 2H, H5 and H6, $J_{5,6}$=9.5 Hz), 6.78 (d, 1, C12-OH, $J_{12,OH}$=3.5 Hz), 6.80 (m, 1, H9), and 7.38 (m, 2, H10 and H11), ^{1}H NMR (Me_2SO) δ 1.01 (d, CH_3-C, J=5.5 Hz), 2.05~2.75 (m, H2-H4, H12a), 3.75 (s, CH_3O), 4.13 (s, 1, C6a-OH), 4.68 (d, 1, H7, $J_{7,OH}$=4.5 Hz), 5.08 (dd, 1, H12, $J_{12,OH}$=3.5 and $J_{12,12a}$=8.5 Hz), 5.16 (d, 1, C7-OH, $J_{7,OH}$=4.5 Hz), 6.21, 6.68 (AB, 2, H5 and H6, $J_{5,6}$=9.5 Hz), 6.53 (d, 1, C12-OH, $J_{12,OH}$=3.5 Hz), 6.86 (dd 1, H9, $J_{9,11}$=2.5 and $J_{9,10}$=7 Hz), and 7.19 (m, 2, H10 and H11), mass spectrum, *m/z* (relative intensity) 342 (1, M), 324 (7, M$-H_2O$), 316 (100, M$-2H_2O$), 291 (58), 263 (10), 236 (17).

Anal. Calcd. for $C_{20}H_{22}O_5$: C 70.16, H 6.48.
Found: C 70.02, H 6.47.

(3*R*) or (3*S*)-3,4-Dihydro-8-methoxy-3-methyl-2*H*-benz[a]anthracene-1,7,12-trione (**3**, X-14881 C)

Component X-14881 C crystallized as yellow prisms. A molecular formula of $C_{20}H_{16}O_4$ was suggested by mass spectrometry and the ^{1}H NMR spectrum revealed the familiar CH_2–CH(Me)–CH_2 moiety, the methoxy group, the *cis* olefin and the three consecutive benzenoid protons as the only absorptions. IR and UV spectra supported the 3,4-dihydro-(2*H*)-benz[a]anthracene-1,7,12-trione chromophore establishing the constitution of **3**.

The substance exhibited Rf 0.73 (system 2), mp>235°C (dec), UV max (chloroform) 376 (ε 5,150), 266 (32,750), and 250 nm (infl., 22,000), IR (KBr) 1705 (Cl=O), 1697 (C12=O), and 1673 cm^{-1} (C7=O), Fig. 1, ^{1}H NMR ($CDCl_3$) δ 1.18 (d, CH_3C, J=5.5 Hz), 2.35~3.15 (m, 5, H2-H4), 4.01 (s, CH_3O), 7.27

(dd, 1, H9, $J_{9,11}$=2.5 and $J_{9,10}$=7.5 Hz), 7.48, 8.24 (AB, H5 and H6, $J_{5,6}$=8 Hz), 7.67 (t, 1, H10, $J_{9,10}$=$J_{10,11}$=7.5 Hz), and 7.76 (dd, 1, H11, $J_{9,11}$=2.5 and $J_{10,11}$=7.5 Hz), mass spectrum, *m/z* (relative intensity) 320 (100, M), 305(16), 292 (28), 289 (7), 278 (64), 261 (21), 249 (39).

[3*R*-(3α,4ξ,6aβ,7β,12α,12aα)] or [3*S*-(3β,4ξ,6aα,7α,12β,12aβ)]-3,4,6a,7,12,12a-Hexahydro-4,6a,7,12-tetrahydroxy-8-methoxy-3-methylbenz[a]anthracen-1(2*H*)-one (**4**, X-14881 D)

The least polar of the five components, **4** was obtained as colorless prisms with a UV spectrum similar to **2** suggesting the same chromophores. The highest mass in the spectrum of **4** was observed at *m/z* 340. In view of the propensity of these materials to generate high-mass peaks by dehydrations, the *m/z* 340 peak was interpreted as M$-H_2O$. X-14881 D, thus suggested to be a hydroxylated derivative of **2**, showed ^{1}H NMR signals (Me_2SO-d_6) in support of structure **4**. In addition to the major spectral features of **2**, the spectrum of **4** exhibited an additional doublet for a HOCH< function. Moreover, the upfield portion of the AB pattern, due to the olefinic H5 observed at δ 6.21 in **2**, was found at δ 6.61 in **4** establishing the position of the new hydroxyl group at C4.

The substance was homogeneous with Rf 0.22 (system 2), mp>160°C (dec), UV max (chloroform) 300 (ε 8,360) and 281 nm (7,260), IR (KBr) 1630 cm^{-1} (hydrogen-bonded, conjugated carbonyl, Fig. 1), ^{1}H NMR (Me_2SO-d_6) δ 1.06 (s, CH_3C), 1.90~2.80 (m, 4, H2, H3 and H12a), 3.81 (s, CH_3O), 4.04 (m, 1, H3), 4.05 (s, 1, C6a-OH), 4.72 (d, 1, H7, $J_{7,OH}$=4 Hz), 5.11 (dd, 1, H12, $J_{12,OH}$=3.5 and $J_{12,12a}$=8.5 Hz), 5.19 (d, 1, C7-OH, $J_{7,OH}$=4 Hz), 5.57 (d, 1, C4-OH, $J_{4,OH}$=7.5 Hz), 6.38 (d, 1, C12-OH, $J_{12,OH}$=3.5 Hz), 6.62, 6.70 (AB, H5 and H6, $J_{5,6}$=10 Hz), 6.87 (dd, 1, H9, $J_{9,11}$=2.5 and $J_{9,10}$=7.0 Hz), and 7.18 (m, 2, H10 and H11), mass spectrum, *m/z* (relative intensity) 340 (4, M$-H_2O$), 322 (100, M$-2H_2O$), 304 (31, M$-3H_2O$), 307 (16), 289 (35), 276 (6).

1-Hydroxy-8-methoxy-3-methylbenz[a]anthracene-7,12-dione (**5**, X-14881 E)

Structure **5** proposed for component X-14881 E is based only on sparse evidence, the substance was obtained as a yellow film, Rf 0.29 (dichloromethane), ^{1}H NMR ($CDCl_3$) δ 2.49 (s, CH_3–C), 4.06 (s, CH_3O), 7.15 (d, 1, H2, $J_{2,4}$=1.5 Hz), 7.39 (d, 1, H4, $J_{2,4}$=1.5 Hz), 7.52 (m, 1, H9), 7.73 (t, 1, H10, $J_{9,10}$=$J_{10,11}$=8 Hz), 7.95 (dd, 1, H11, $J_{9,11}$=1.5 and $J_{10,11}$=8 Hz), 8.11, 8.30 (AB, H5 and H6, $J_{5,6}$=9 Hz).

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