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Note

Identification of new elloramycins, anthracycline-like antibiotics, in biological cultures by high-performance liquid chromatography and diode array detection

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The anthracycline antibiotic elloramycin is produced by *Streptomyces olivaceus* TÜ 2353, and was detected in the course of a chemical screening by thin-layer chromatography (TLC)[1]. The search for new anthracycline antibiotics is based on the requirement for less toxic substances in antitumour therapy. This can be achieved by the isolation of new structures or analogues, or by derivatization. Anthracycline-producing organisms often produce more than one compound of these yellow and red pigments, as in case of aclacinomycins[2,3], rhodirubins[4], baumycins[5], roseorubicins[6], tetracenomycins[7], doxorubicins[8], auramycins and sulphurmycins[9].

As reported previously, diode array detection allows the classification of structure-related compounds in comparing the UV–visible spectra of peaks during high-performance liquid chromatography (HPLC)[10]. Since anthracycline antibiotics show a characteristic UV–visible spectrum, it should be possible to detect minor congeners as well as the main compound.

EXPERIMENTAL

Chemicals

Acetonitrile (HPLC grade) and orthophosphoric acid (analytical grade) were obtained from Merck (Darmstadt, F.R.G.). Water was purified by means of a Milli-Q system (Millipore, Eschborn, F.R.G.).

Chromatographic system

A HP-1090A liquid chromatograph (Hewlett-Packard, Waldbronn, F.R.G.) was used with a DR5 solvent-delivery system, variable-volume auto-injector, auto-sampler and HP-1040A diode array detection system. A detection wavelength of 288 nm was used and the ratio of wavelength to bandwidth was 288:4 nm. Detector signals were processed and recorded on an HP-3392A recording integrator. Signals and spectra were stored on an HP-9121 flexible disk drive and plotted with an HP-7470A plotter.

Columns

The column (125 × 4.6 mm I.D.) was fitted with a guard column (20 × 4.6

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mm I.D.) and filled with 5 μm Shandon ODS Hypersil; it was obtained from Bischoff (Leonberg, F.R.G.).

Mobile phases

The biological samples were separated by gradient elution. Solvent A was 0.1% phosphoric acid, solvent B was acetonitrile. The linear gradient was from 30% to 70% solvent B in 8 min, and the flow-rate was 2 ml/min.

Sample preparation

The fermentation broth was centrifuged, and 20 ml of the supernatant were adsorbed onto an Extrelut-20 column (Merck). The lipophilic compounds were eluted with dichloromethane, concentrated *in vacuo* and resuspended in 2 ml of methanol. The HPLC injection volume was 10 μl.

RESULTS

The dichloromethane extract of the culture filtrate from the fermentation broth of *Streptomyces olivaceus* was investigated by HPLC and diode array detection, as shown in Fig. 1. Besides the main peak of the elution profile with a retention time of 6.97 min, which represents elloramycin, five minor peaks with retention times between 4 and 8 min, labelled B, C, D, E and X, could be detected.

Comparison of the UV–visible spectra of elloramycin with these minor peaks, as shown in Fig. 2, indicates the presence of new structure-related compounds. The maxima of 287 nm in the UV region and of 390 nm and 410 nm in the visible region are identical with those of elloramycin.

The compounds C, D and E were isolated from the fermentation broth by

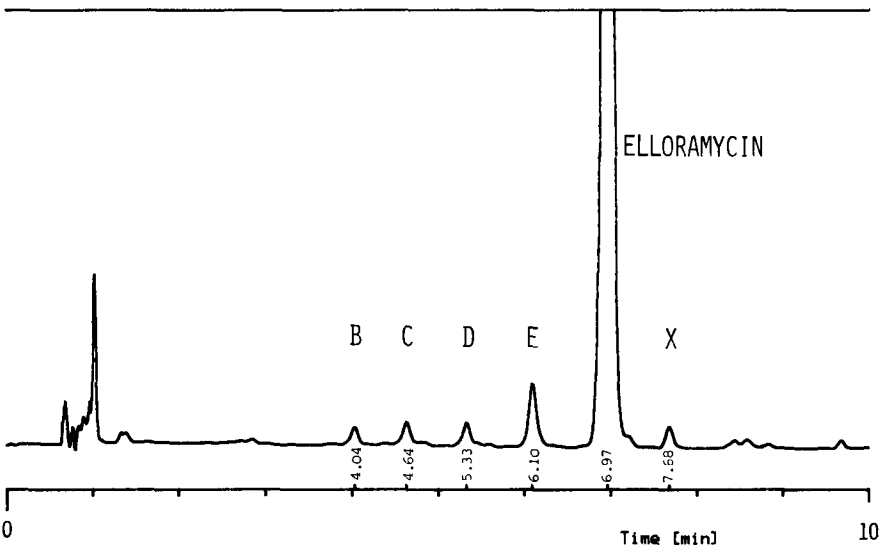


Fig. 1. HPLC chromatogram of a dichloromethane extract from the culture filtrate of *S. olivaceus*, plotted at 280 nm, attenuation 0.1 a.u.f.s.

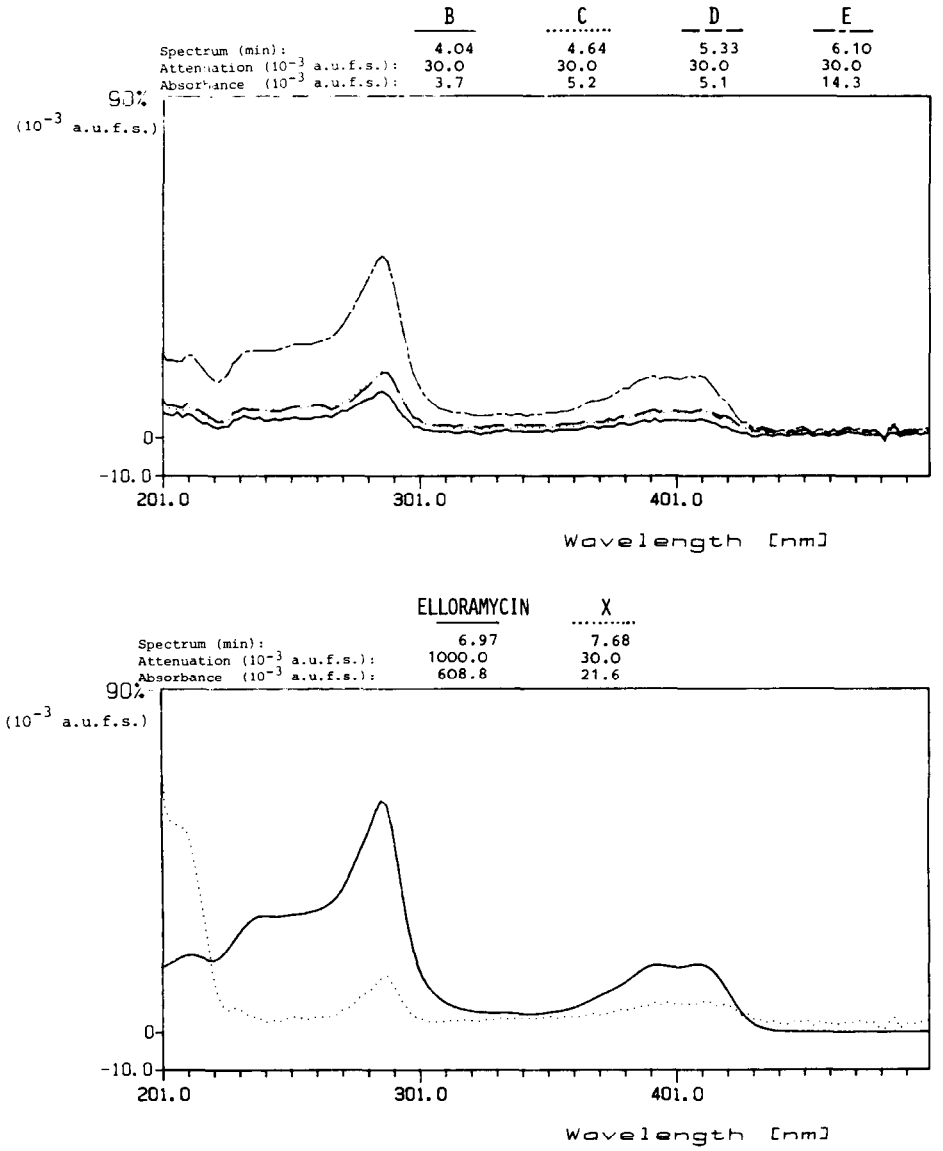


Fig. 2. UV–visible spectra of elloramycin and compounds B, C, D, E and X, recorded during HPLC run.

chromatography on Amberlite XAD-2, dichloromethane extraction, chromatography on silica gel and preparative HPLC on Hypersil ODS. Compound B was separated by chromatography on silica gel[11]. The concentration of elloramycin in the fermentation broth was 166 mg/l, those of compounds B, C, D and X were 1.3 mg/l and that of compound E was 4 mg/l. Compound X could not be isolated in sufficient amounts for chemical investigations. Structure elucidation confirmed the assumption of new elloramycin structures, which are shown in Fig. 3. The chemical and biological data are reported elsewhere[11].

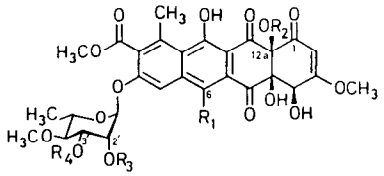
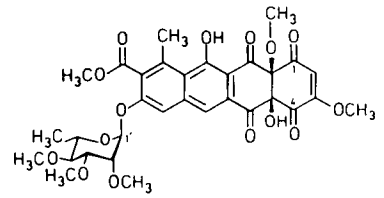
COMPOUND		R_1	R_2	R_3	R_4
ELLORAMYCIN		H	CH_3	CH_3	CH_3
ELLORAMYCIN B		H	H	H	CH_3
ELLORAMYCIN C		H	CH_3	CH_3	H
ELLORAMYCIN D		H	CH_3	H	CH_3
ELLORAMYCIN E					

Fig. 3. Structures of elloramycin and the new elloramycin compounds.

CONCLUSIONS

TLC lacks power in the detection of minor congeners when their concentration is between 0.7% and 2.5% of that of the main compound, as in case of the new elloramycins. Screening for structure-related substances using HPLC and diode array detection seems to be more efficient, because the UV–visible spectra of each peak of the chromatogram can be compared with that of the parent compound. However, a limitation is the signal intensity, because values less than $5 \cdot 10^{-3}$ a.u.f.s. do not allow reliable graphical reproduction. In case of elloramycins the limit is reached at a concentration of *ca.* 1 mg/l.

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