Synthesis of the Antibiotic Anticapsin[1]

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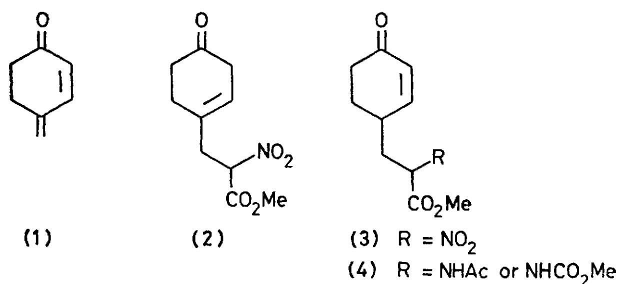
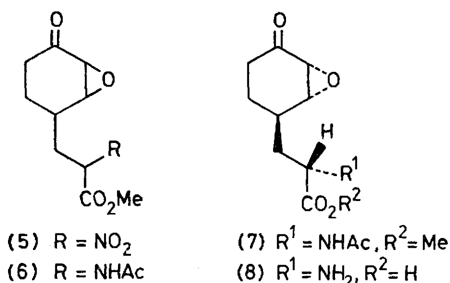
Summary The antibiotic anticapsin (**8**) has been synthesised by a route involving Michael addition of methyl nitro-acetate to 4-methylenecyclohex-2-enone (**1**) and enzymic deacylation of the derived (±)-*N*-acetylanticapsin methyl ester (**7**).

ANTICAPSIN, an amino-acid antibiotic from *Streptomyces griseoplanus*[2] and *Bacillus subtilis*[3] which inhibits hyaluronic acid capsule formation in *Streptococcus pyogenes*,[2] has been assigned[3,4] the structure and absolute stereochemistry (**8**). It occurs also as the *C*-terminal amino-acid in bacilysin,[3] a substance formed by certain bacteria which causes lysis in growing staphylococci and which has been shown to be identical with tetaine and bacillin.[5] We report here a synthesis of anticapsin.

Synthetic approaches utilising reduced tyrosine derivatives of type (**4**) were precluded by the ease of intramolecular addition of the amino-function to the conjugated ketone to give 1,2-disubstituted-6-oxo-octahydroindoles.[6] Accordingly the carbon sleleton of anticapsin was generated by Michael addition of methyl nitro-acetate to the exocyclic double bond of the known[7] 4-methylenecyclohex-2-enone (**1**), catalysis by *N*-benzyltrimethylammonium hydroxide affording the adduct (**2**) in 95% yield. Equilibration of the $\beta\gamma$-enone (**2**), ν_{max} (film) 1753 (CO_2Me), 1717 (CO), and 1560 cm^{-1} (NO_2), $\delta(CDCl_3)$ 5·64 (1H, m, =CH), 5·32 (1H, dd, *J* 6 and 10 Hz, $CHNO_2$), and 3·84 (3H, s, CO_2Me), with 1% hydrochloric acid in dimethyl sulphoxide at room temperature gave a 1:1 mixture, separable by silica gel chromatography, of the $\beta\gamma$- and $\alpha\beta$-enones. The $\alpha\beta$-enone (**3**) (a 1:1 mixture of diastereoisomers), ν_{max} (film) 1750 (CO_2Me), 1675 (conjugated CO), and 1555 cm^{-1} (NO_2), $\delta(CDCl_3)$ 6·78 [1H, m, C(3)-H], 6·00 [1H, dd, *J* 10 and 2 Hz, C(2)-H], 5·32 (1H, m, $CHNO_2$), and 3·84 (3H, s, CO_2Me), was oxidised with alkaline hydrogen peroxide in methanol at −20 °C to form the $\alpha\beta$-epoxyketone (**5**) (79%), ν_{max} (film) 1750 (CO_2Me), 1715 (CO), and 1560 cm^{-1} (NO_2), $\delta(CDCl_3)$ 5·30 (1H, m, $CHNO_2$), 3·84 (3H, s, CO_2Me), 3·45 [1H, m, C(3)-H], and 3·25 [1H, d, *J* 4 Hz, C(2)-H]. The product (**5**) was a mixture of stereoisomers, but was too unstable to allow separation at this stage.

Catalytic reduction of the nitro-group in (**5**) with Raney nickel in acetic anhydride gave the *N*-acetylamino-epoxyketone (**6**) (35%), readily separated by silica gel chromatography into *cis* and *trans* components (as regards the epoxy and alanyl substituents) in 4:1 ratio. The two *cis* diastereoisomers (1:1 ratio), $\delta(CDCl_3)$ 3·55 [dd, *J* 4 and 2 Hz, C(3)-H] and 3·23 [d, *J* 4 Hz, C(2)-H], and $\delta(CDCl_3)$





3·41 [dd, J 4 and 2 Hz, C(3)-H] and 3·21 [d, J 4 Hz, C(2)-H], were readily distinguished from the two more polar *trans* diastereoisomers (1 : 1 ratio), $\delta(CDCl_3)$ 3·74 [d, partly obscured by CO_2Me, C(3)-H] and 3·25 [d, J 4 Hz, C(2)-H], and $\delta(CDCl_3)$ 3·41 [d, J 4 Hz, C(3)-H] and 3·22 [d, J 4 Hz, C(2)-H], by the vicinal coupling constant of 2 Hz between C(3)-H and C(4)-H in the *cis* compounds, in contrast to the virtual absence of any such coupling in the *trans* compounds.[4,8]

Careful silica gel chromatography separated the two *trans* diastereoisomers of the *N*-acetylamino-epoxy-ketone (**6**). The more polar isomer showed identical ^1H n.m.r. characteristics, $\delta(CDCl_3)$ 6·04 (1H, brs, exchangeable NH), 4·77 (1H, m, C*H*NHAc), 3·77 (3H, s, CO_2Me), 3·41 [1H, d, J 4 Hz, C(3)-H], 3·22 [1H, d, J 4 Hz, C(2)-H], and 2·06 (3H, s, COMe), to *N*-acetylanticapsin methyl ester prepared[4] from authentic anticapsin, and accordingly has the relative stereochemistry represented in (**7**), corresponding to that of (−)-anticapsin (**8**).

Conversion of (±)-*N*-acetylanticapsin methyl ester (**7**) into anticapsin was accomplished by alkaline hydrolysis of the methyl ester (1·25 M aqueous sodium hydroxide, 1 mol. equiv., 5 °C, 15 min), followed by enzymic *N*-deacetylation with hog kidney acylase† (38 °C, pH 7, 16·5 h). A relatively high ratio (2 : 1) of enzyme : substrate was required. The product, isolated in 9% yield (based on utilisation of one enantiomer) after gel filtration and cellulose chromatography, was identical to natural (−)-anticapsin (**8**) by amino-acid analysis, t.l.c. in two solvent systems, and ^1H n.m.r. spectroscopy, δ (D_2O, relative to external Me_4Si) 4·36 (t, J 7 Hz, C*H*NH_2), 4·20 [d, J 4 Hz, C(3)-H], and 3·87 [d, J 4 Hz, C(2)-H]. Comparison of c.d. spectra of this product and authentic anticapsin indicated a content of 87% of the natural (−)-enantiomer (**8**). In view of the limited quantity of material available for chiroptical measurements, and the virtually exclusive preference of the renal acylases for L-α-amino-acid substrates,[9] the enzymic deacylation is probably enantiospecific.

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† Calbiochem salt-free lyophilised B grade.

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