

ALBORIXIN, A NEW ANTIBIOTIC IONOPHORE: ISOLATION, STRUCTURE, PHYSICAL AND CHEMICAL PROPERTIES

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Alborixin, a polycyclic polyether ionophorous antibiotic, active against Gram-positive bacteria and fungi, was isolated from cultures of a strain of *Streptomyces albus*. The isolation, structure and physicochemical properties of this antibiotic are reported. Some derivatives have been prepared and their structures and properties are also described in this paper.

In the course of our screening for new antibiotics, a new compound with a broad antibacterial and antifungal spectrum, but also a high toxicity, has been isolated from cultures of a strain of *Streptomyces albus*. This antibiotic, which we have named alborixin, is a polycyclic polyether with an acidic function. Its structure has been elucidated by X-ray crystallography of its potassium salt.¹⁾ In this paper, the isolation, structure and physico-chemical properties of this antibiotic are described.

The description, morphologic study of the producing strain and the spectrum of activity of alborixin will appear in the separate paper.²⁾

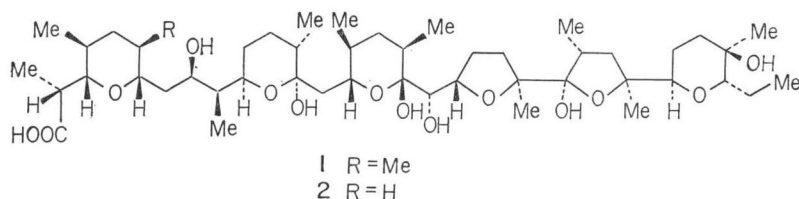
Production and Isolation

The strain was grown in a 20-liter fermentor and the antibiotic was extracted from the mycelium. A schematic representation of the isolation process is shown in Fig. 1. The details are described in the Experimental section.

Structure and Physical Properties of Alborixin and its Potassium Salt

Alborixin is a polycyclic polyether bearing an acidic function, with molecular formula $C_{48}H_{84}O_{14}$ whose structure (1) is closely related to that of X-206 (2). This group of ionophorous polyethers is now composed of 14 antibiotics which are: monensin,³⁾ nigericin,⁴⁾ lasalocid⁵⁾ or X-537 A, grisorixin,⁶⁾ X-206,⁷⁾ dianemycin,⁸⁾ A 204 A,⁹⁾ salinomycin,¹⁰⁾ A 23187,¹¹⁾ septamycin,¹²⁾ lysocellin,¹³⁾ and more recently Ro 21-6150¹⁴⁾ and lonomycin¹⁵⁾ or emericid.¹⁶⁾

Alborixin is a white solid, very soluble in organic solvents, but insoluble in water, which is



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Fig. 1. Isolation and purification of alborixin

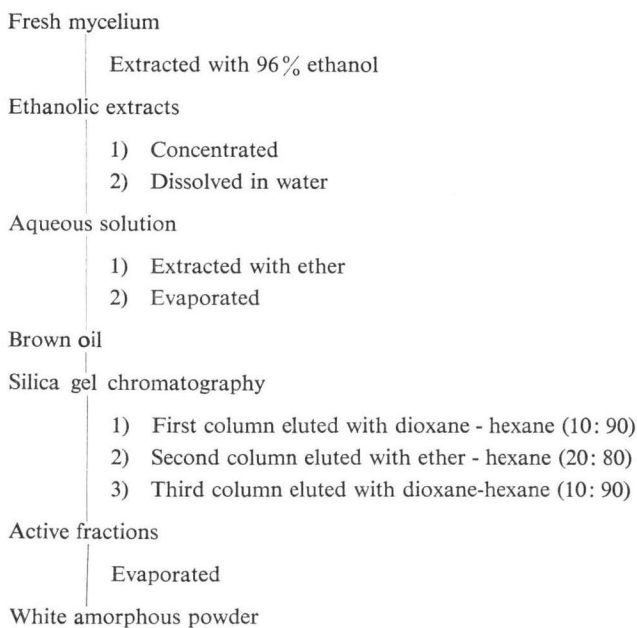
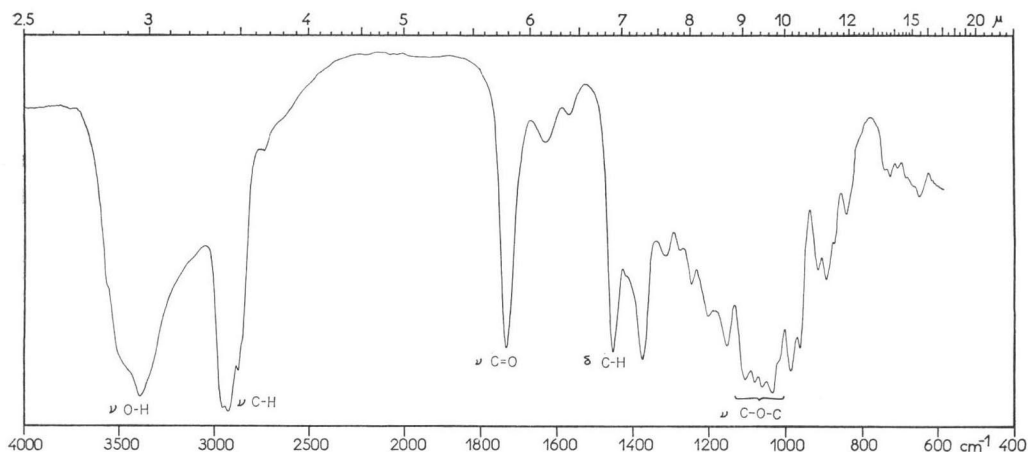


Fig. 2. IR spectrum of alborixin (KBr)



easily isolated as an amorphous powder, m.p. 100~105°C, $[\alpha]_{D}^{20} - 7^\circ$ (*c* 4, acetone). Its pK, determined at 25°C in methanol, is 10.02.

The IR spectrum in KBr (Fig. 2) shows OH functions (wide band between 3700 and 3100 cm^{-1}), a carboxyl C=O (intense band at 1725 cm^{-1}) and several maxima between 1100 and 1030 cm^{-1} due to C-O-C bonds.

The NMR spectrum of the antibiotic (Fig. 3) in CDCl_3 shows two singlets at 6.01 and 6.46 ppm due to the protons of two hydroxyl functions which disappear when D_2O is added. The acidic proton does not appear on the spectrum probably because it participates in an intramolecular hydrogen bond.

Lastly, the mass spectrum of alborixin shows a fragmentation pattern very similar to that observed for nigericin or grisorixin.⁹⁾ The main fragment ions are due to cleavages of the C-C bonds between

the rings or hydroxyl α cleavages (Fig. 4). The molecular ion peak is absent, the highest mass ion peak observed corresponding to the loss of three water molecules from the parent ion. This ion ($M^+ - 3H_2O$) then loses 3 additional water molecules.

We have prepared several monovalent cation salts of alborixin (Na^+ , K^+ , Rb^+ , Cs^+ , Ag^+ , Tl^+) but only the potassium salt has been obtained crystallized: $C_{48}H_{88}O_{14}K$, m.p. 209~210°C. It is a complexed salt whose conformation is similar to those of the corresponding ligand-cation complexes obtained with the other ionophores of the same group. However, in this case, there are 3 intramolecular hydrogen bonds which stabilize the cyclic conformation of the complex (Fig. 5). The cation is coordinated to 8 oxygen atoms of the hydrocarbon chain.

The mass spectrum of this salt shows the molecular ion peak at m/e 923 (2.3% of the base peak) and a peak at m/e 879 corresponding to the loss of CO_2 from the parent ion. It is not possible to show the same fragmentation pattern as in the case of the potassium salts of monensin,¹⁷⁾ nigericin,¹⁷⁾ or grisorixin¹⁸⁾ since this mass spectrum shows a lack of peaks in the high mass area.

The ^{13}C -NMR spectrum of the potassium salt appears in Fig. 6 and the chemical shifts of the signals

Fig. 3. 1H -NMR spectrum of alborixin ($CDCl_3$, 270 MHz)

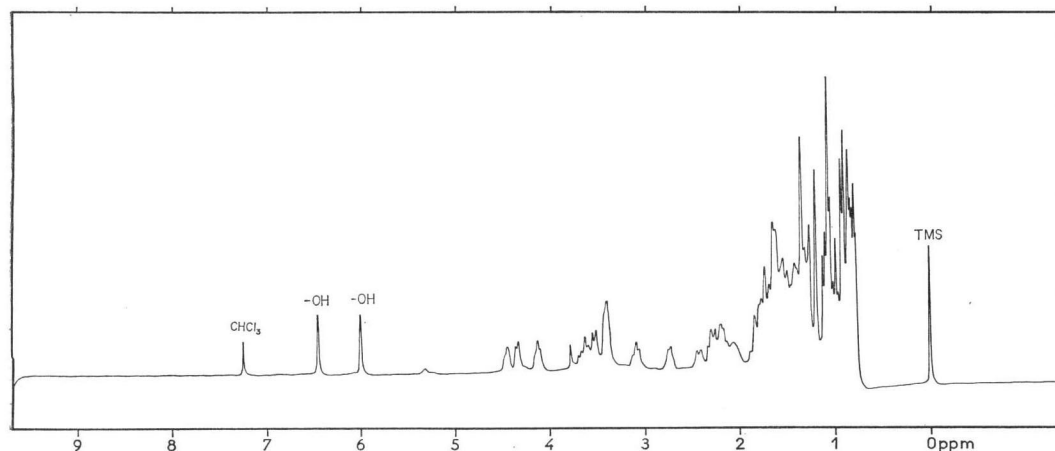


Fig. 4. Mass spectral fragmentation of alborixin

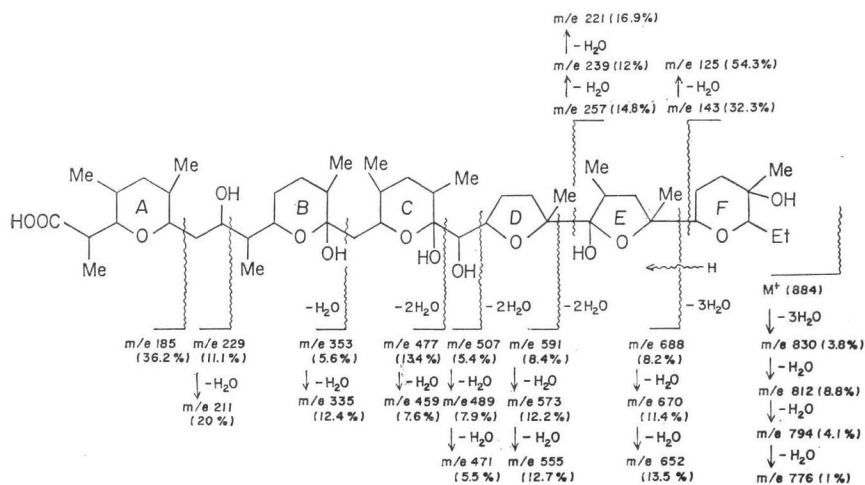
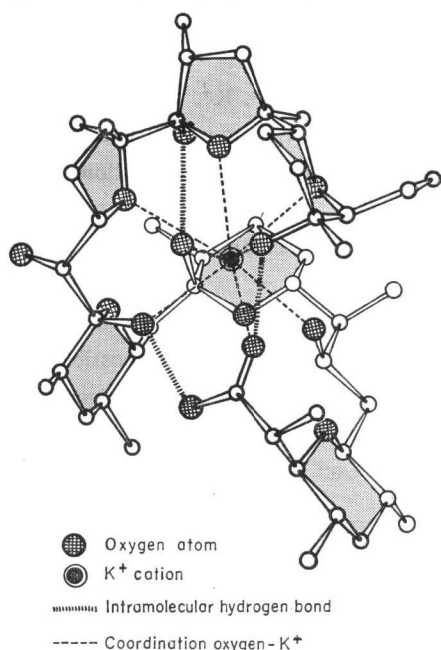


Fig. 5. Structure of the K⁺ salt of alborixin

are given in Table 1. The signal 45 which appears at 180.7 ppm is due to the carboxyl carbon. Between 67.7 and 108.4 ppm, 15 signals are present which correspond to the carbons bonded to at least one oxygen atom. The off resonance spectrum shows that signals 30,38,41, 42,43 and 44 are due to the 6 quaternary carbons of the molecule. Signals 42,43 and 44 probably correspond to the 3 hemiacetal carbons. Lastly, among the signals which appear between 0 and 46 ppm, peaks 2,6 and 12 are probably double.

Table 1. ¹³C Chemical shifts for K⁺ salt of alborixin (from (CH₃)₄Si)

Signal No.	δ (ppm)	Signal No.	δ (ppm)
1	8.6	25	39.6
2	10.3	26	42.1
3	16.1	27	42.3
4	16.7	28	42.7
5	17.0	29	46.0
6	17.5		
7	18.5		
8	19.8	30	67.7
9	20.1	31	68.7
10	24.1	32	69.7
11	26.6	33	69.9
12	26.9	34	71.5
13	27.9	35	72.4
14	30.5	36	78.6
15	31.0	37	82.5
16	32.3	38	83.3
17	33.5	39	85.7
18	33.8	40	86.4
19	34.9	41	90.0
20	35.2	42	98.2
21	36.5	43	99.4
22	38.2	44	108.4
23	38.8		
24	39.1	45	180.7

Chemical Study of Alborixin

We have prepared several derivatives of alborixin, all biologically inactive. The treatment of alborixin with an ethereal solution of diazomethane affords the methyl ester: C₄₀H₈₀O₁₄, m.p. 67~68°C. Acetylation with acetic anhydride in pyridine yields a triacetate, C₅₄H₉₀O₁₇, m.p. 70~75°C to which it has not been possible to assign a structure. Its mass spectrum does not exhibit a molecular ion peak, rather peaks at *m/e* 974 (M⁺-2H₂O) and *m/e* 956 (M⁺-3H₂O).

By trimethylsilylation of alborixin with trimethylsilyl-chloride-disilazane, we obtained a tetrasilylated derivative: C₆₀H₁₁₆O₁₄Si₄. The acidic function was silylated as well as 3 undetermined hydroxyl groups. The mass spectrum shows a peak at *m/e* 1137 (M⁺-3H₂O).

The oxidation by KIO₄ of the two vicinal hydroxyl groups between rings C and D failed probably because of the trans conformation of these two hydroxyl functions and their considerable steric hindrance.

The reduction of alborixin with NaBH₄ in ethanol affords a mixture of two reduced derivatives which were assigned structures (3) and (4). (Fig. 7). Compound 3, C₄₈H₈₈O₁₄ (M=888) represents

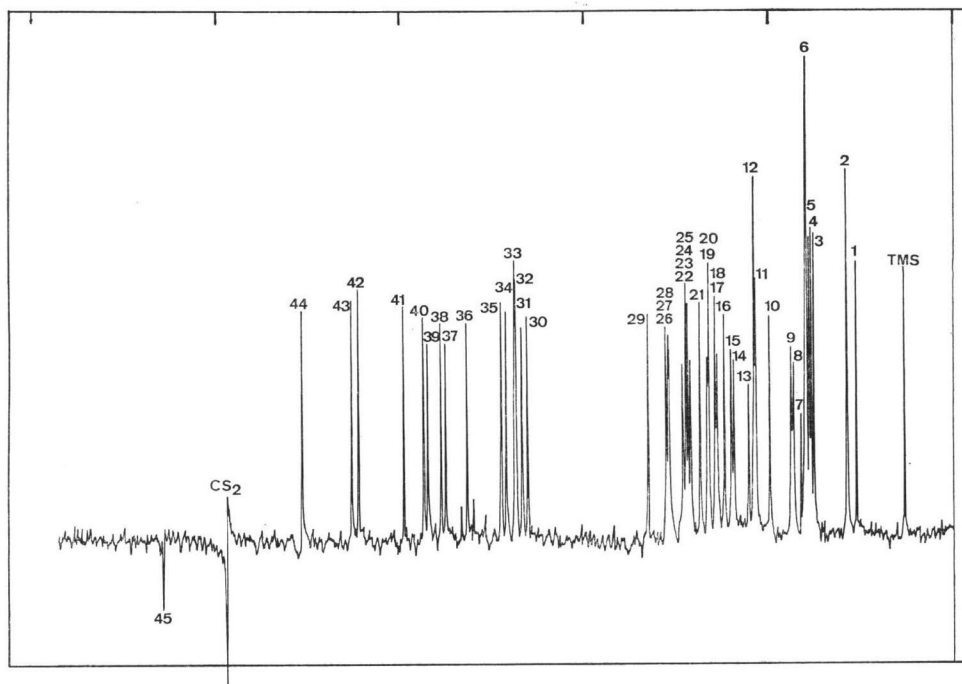
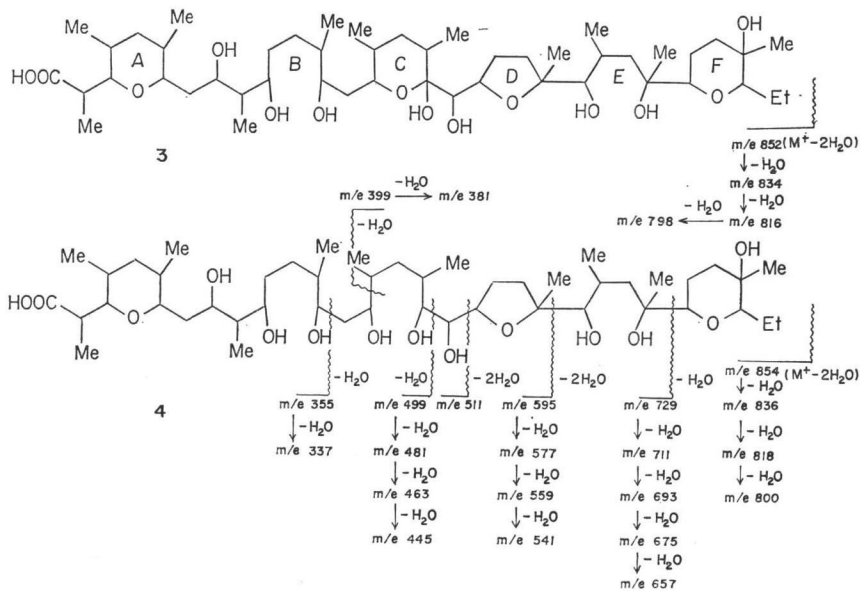
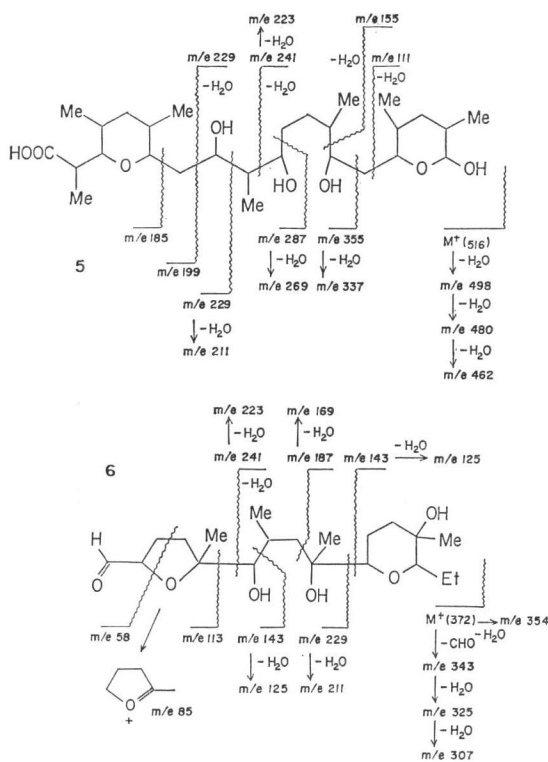
Fig. 6. ^{13}C -NMR spectrum of alborixin

Fig. 7. Reduction products of alborixin



only 10% of the mixture. The two hemiacetal rings *B* and *E* have been reduced. Its mass spectrum confirms this structure. The molecular ion peak is not present but a peak appears at m/e 852 due to the loss of two water molecules from the parent ion. Compound 4 of molecular formula $\text{C}_{48}\text{H}_{90}\text{O}_{14}$ ($M=890$) bears 9 hydroxyl groups, the 3 hemiacetal rings *B*, *C* and *E* being reduced. Its mass spectrum is similar to that of compound 3 and is described in Fig. 7.

Fig. 8. Oxidation products of derivative 4



The oxidation by KIO_4 of compound 4 in ethanol affords two derivatives which were assigned structures 5 and 6 (Fig. 8). Compound 5 has a molecular formula $C_{28}H_{52}O_8$ ($M=516$). Its 1H -NMR spectrum does not show an aldehydic proton, so we think that the aldehyde previously formed gives the more stable cyclic hemiacetal 5. The mass spectrum of this compound described in Fig. 8 confirms this structure.

The NMR spectrum of compound 6, $C_{20}H_{38}O_6$ ($M=372$) shows a doublet ($J=3Hz$) at 9.6 ppm corresponding to an aldehydic proton coupled with another proton. The mass spectrum of this compound is described in Fig. 8.

Experimental

General

Melting points (m.p.) were determined with a Reichert microscope and are uncorrected. 1H -NMR spectra were determined either with a Perkin-Elmer R-24 or a Jeol C-60 HL spectrometer with TMS as the internal reference. The 1H -NMR spectrum of alborixin was measured with a Bruker 270 MHz equipped with a Nicolet 1085 computer. The ^{13}C -NMR spectrum of the potassium salt of alborixin was determined with a Jeol FX-60 spectrometer and TMS was used as internal standard. Mass spectra were taken with a Varian MAT CH 5 spectrometer at 70 eV. IR spectra were measured on a Perkin-Elmer 180 instrument. Column chromatography was performed on Merck silica gel (0.063~0.200 mm). Analytical tlc plates were Merck (silica gel 60) and preparative tlc plates were prepared with Merck silica gel PF₂₅₄₊₃₀₀.

Fermentation and Isolation of Alborixin

Growth of the organism will be described in the separate paper.²⁾ The strain was grown in a 20 liter fermentor and the incubation was carried out for 4~7 days at 27°C.

The mycelium was filtered, dried, and extracted with 96% ethanol (5 ml of ethanol per g of fresh mycelium). The ethanolic extracts were then concentrated, evaporated to dryness and dissolved in water. The aqueous solution thus obtained was extracted with ether, the ethereal extracts being dried over Na_2SO_4 and evaporated to dryness. A thick, brown oil was obtained which was then chromatographed on silica gel columns. Three successive columns were necessary to obtain the pure antibiotic: The first column was eluted with a dioxane - hexane mixture (10: 90) with the percentage of dioxane gradually increasing to 20% at the end of the run. The active fractions were concentrated and chromatographed on the second column. Elution was begun with an ether - hexane mixture (20: 80), the percentage of ether gradually increasing to 50% at the end of the run. The active fractions were then chromatographed a third time using the same conditions as for the first column. Pure antibiotic was thus isolated as a white solid foam after the eluant had been evaporated under vacuum.

Alborixin had a tlc Rf of 0.40 on Merck silica gel sheets using an ethyl acetate - cyclohexane mixture (40: 60) for development and a Rf of 0.50 with a dioxane - hexane mixture (20: 80).

Alborixin exhibited a red color when treated with 50% H_2SO_4 at 110°C on tlc plates.

The average yield of antibiotic was 350 mg per liter of medium.

Preparation of the Potassium Salt of Alborixin

To a solution of 1.14 g of alborixin in 50 ml of 96% ethanol was added 0.1 N KOH to pH 8.5 (pH meter). After evaporation at room temperature for 2 days, white crystals precipitated. These crystals were filtered and recrystallized from methanol - water (50:50) by evaporation at room temperature. Potassium salt (1.04 g) was obtained. IR $\nu_{\text{max}}^{\text{KBr}}$: 1560 cm^{-1} ($-\text{COO}^-$). NMR (CDCl_3 , 270 MHz): the two hydroxyl singlets shown on the NMR spectrum of alborixin at 6.01 and 6.46 ppm were shifted to 5.54 and 6.86 ppm in the spectrum of the potassium salt.

Calcd. for $\text{C}_{48}\text{H}_{88}\text{O}_{14}\text{K}$: C 62.44, H 9.06, K 4.24
 Found: C 62.73, H 9.02, K 4.25

Methyl Ester

Alborixin (300 mg) was treated with 10 ml of ethereal diazomethane. After one night at room temperature, the mixture was evaporated to dryness and the residue chromatographed on a preparative tlc plate. The methyl ester was thus obtained: Rf 0.60 with an ether - hexane mixture (50:50). m.p. 67~68°C. IR $\nu_{\text{max}}^{\text{KBr}}$: 1740 cm^{-1} ($-\text{CO}$ ester). NMR (CCl_4) δ ppm: 3.65 (3H,s,methyl ester), 5.75 (1H,s,-OH), 6.25 (1H,s,-OH).

Calcd. for $\text{C}_{40}\text{H}_{86}\text{O}_{14}$: C 65.45, H 9.64, O 24.91
 Found: C 65.67, H 9.60, O 24.42

Acetylation

Alborixin (130 mg) was treated with 5 ml of an acetic anhydride - pyridine mixture (20:80) for one hour at 70°C. After dilution in 100 ml of water, the aqueous medium was extracted with ether, the etherous extracts were dried over Na_2SO_4 and evaporated to dryness. The triacetate was purified by preparative tlc with an ether - hexane (80:20) eluant. The triacetate (80 mg) was thus isolated as a white solid: Rf 0.30 with the eluant above. m.p. 70~75°C. IR $\nu_{\text{max}}^{\text{KBr}}$: 1740 cm^{-1} (intense band C=O), 1250 cm^{-1} (C-O-C). NMR (CCl_4) δ ppm: 1.90 (3H,s,-OAc), 1.98 (3H,s,-OAc), 2.07 (3H,s,-OAc)

Calcd. for $\text{C}_{54}\text{H}_{90}\text{O}_{17}$: C 64.13, H 8.98, O 26.89
 Found: C 64.14, H 8.82, O 26.94

Trimethylsilylation

Alborixin (100 mg) dissolved in 5 ml of pyridine was treated at room temperature with 1 ml of trimethylsilyl-chloride and 3 ml of disilazane. After 15 minutes, the mixture was evaporated to dryness, the residue dissolved in benzene, filtered and evaporated again. The silylated derivative (70 mg) was isolated by preparative tlc with an ether - hexane mixture (20:80): Rf 0.40 with the same eluant. NMR (CCl_4): several signals near 0 ppm due to the trimethylsilyl groups. This derivative was quite unstable and did not give a good elementary analysis.

Reduction of Alborixin

Alborixin (1 g) was stirred for 24 hours at room temperature with 2 g of NaBH_4 in 10 ml of ethanol. The mixture was then filtered and the filtrate evaporated to dryness. The residue was chromatographed on a silica gel column with a chloroform - methanol mixture (90:10), affording 750 mg of compound 4: m.p. 81~82°C. Rf 0.45 with the eluant above.

Calcd. for $\text{C}_{45}\text{H}_{90}\text{O}_{14}$: C 64.68, H 10.18, O 25.13
 Found: C 64.29, H 10.26, O 24.00

Compound 3 has not been isolated really pure.

To 1.5 g of compound 4 dissolved in 10 ml dioxane was added a solution of 3 g of KIO_4 in water. After standing 3 days at room temperature, the mixture was filtered and the filtrate evaporated to dryness; the residue was dissolved in CHCl_3 and the solution filtered again. After evaporation of CHCl_3 , the residue was chromatographed on preparative tlc sheets with a chloroform - methanol mixture (90:10). Three constituents were isolated: compound 4 (300 mg), compound 5 (180 mg) compound 6 (360 mg). We did not obtain a satisfactory elementary analysis for compound 5. $\text{C}_{28}\text{H}_{52}\text{O}_8$. m.p. 49~50°C. IR $\nu_{\text{max}}^{\text{KBr}}$: 1725 cm^{-1} (carboxyl).

Compound 6 was isolated as a white solid: m.p. 55~56°C. IR $\nu_{\text{max}}^{\text{KBr}}$: 1735 cm^{-1} ($-\text{CHO}$).

Calcd. for $C_{20}H_{30}O_6$: C 64.49, H 9.74, O 25.77
Found: C 64.32, H 9.69, O 25.92

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