As previously oberved with other compounds of the fusicoccin series *O*-acetylated in the glucose moiety[3], compounds X and XI are interconvertible at basic pH values. The phytotoxicity of both compounds (determined by Professor A. GRANITI, University of Bari) is lower than that of fusicoccin[10].

Riassunto. Due nuovi metaboliti minori del fungo fitopatogeno *Fusicoccum amygdali* Del. vengono identificati come 12-*O*-acetilfusicoccina (X) e 12-*O*-acetilisofusicoccina (XI).

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Isolation of β-Ecdysone (20-Hydroxyecdysone) from the Parasitic Nematode *Ascaris lumbricoides*

The growth of nematodes involves a series of moults[1,2] similar to those marking the successive stages of arthropod development. It is generally accepted that these two widely divergent groups are likely to have evolved from a common ancestral stock. Thus it is of especial interest to establish whether moulting in nematodes is controlled by steroidal moulting hormones like the arthropod ecdysones, or by any similar steroids.

Free moulting hormones occur in insects at such low concentrations that large amounts of tissue are required for satisfactory isolation and characterization of the active substances. The highest concentrations of ecdysones appear in insects at the time of moulting (about 500 μg/kg), whereas during intermoult periods the amounts are much less (about 2 μg/kg)[3]. In the case of nematodes similar concentrations might be expected if ecdysones have a hormonal role.

The parasitic nematode *Ascaris lumbricoides* can be obtained in relatively large numbers from the intestines of pigs. Adults of both sexes, but of unknown age from the final moult, were thoroughly washed in water after collection. Pooled samples were stored frozen until analyzed for the presence of ecdysones. All equipment used for the subsequent extraction and purification procedures was carefully cleaned to avoid contamination of the *Ascaris* extracts with ecdysones of plant origin. The animals (15.5 kg) were thawed overnight and minced into ethanol (70 l). After 24 h at about 20 °C the alcohol was decanted and the residue extracted 3 times further with ethanol (50 l). The combined ethanol extracts were evaporated to an aqueous ethanol extract (11 l) which was twice extracted with hexane (1 l) to remove lipids. The aqueous ethanol phase was then concentrated to 3.5 l, water (3 l) added and the mixture extracted twice with *n*-butanol (3 l). The butanol extracts were combined, evaporated to dryness and partitioned between chloroform (2 l), methanol (3 l) and water (2 l) (2 tube separation). The combined methanol layers were evaporated to an aqueous residue of 750 ml, potassium hydrogen carbonate (20 g) added and the mixture extracted 3 times with an equal volume of butanol. The butanol extracts were in turn washed with an equal volume of water and the combined butanol extracts evaporated to an oily residue (5.6 g). Exploratory studies with this material indicated that ecdysones, if present, were too low in concentration to be detectable with the *Calliphora* bioassay[4]. Therefore to select fractions in the subsequent fractionation procedures which might contain β-ecdysone, tritium labelled β-ecdysone (370×10^3 cpm, 0.185 μg) was added. The labelled β-ecdysone was prepared by injecting [23, 23, 24, 24,-$^3H_4$]-α-ecdysone[5] into 3rd instar larvae of *Calliphora stygia* at the time of puparium formation and isolating the labelled β-ecdysone formed[6].

Further fractionation of the butanol extract by reversed phase partition chromatography[7] afforded a β-ecdysone fraction (64 mg) which was chromatographed on a column of silica gel (10 g, 15% water) made up and eluted with 96% ethanol-chloroform (14:86). The fractions containing the radioactivity were combined and chromatographed on CM-Sephadex[7]. It was then found on plotting the UV-absorption (at 254 nm) and the radioactivity against elution volume that the peak due to the radioactivity coincided with the peak due to UV-absorption. When the peak fractions were chromatographed on a column of silica gel (10 g, 15% water) made up and eluted with 96% ethanol-chloroform (10:90) the UV- and radioactivity curves again coincided closely. From the intensity of the UV-absorption λ *max* 242 nm in water) it was estimated that a total of 4.5 μg of ecdysone was present. When the material isolated was dissolved in 400 μg of water and bioassayed[4] in *C. stygia*, 73% sclerotization was found. These data indicate that a moulting hormone active substance, almost certainly β-ecdysone is present in the extract. Inokosterone, the only other known animal ecdysone with similar chromatographic properties is much less active in the *Calliphora* test[8]. α-Ecdysone could not be detected in the *Ascaris* extracts after similar fractionation steps.

While it is thus established that the *Ascaris* sample contained β-ecdysone, the amount isolated was exceed iongly small (0.3 μg/kg) and is about 1/10 the amount isolated from crayfish at an intermoult stage[9]. It is

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possible that the β-ecdysone isolated was not elaborated by the nematode but was obtained from vegetable material ingested by the parasite from the intestine of the host animal. However, this seems unlikely and the possibility that ecdysones play a role in nematode development now warrants further investigation.

Résumé. Une hormone de mue des arthropodes, très probablement la β-ecdysone, a été trouvée dans un extrait du nématode parasite *Ascaris lumbricoides*, obtenus des intestins du cochons.

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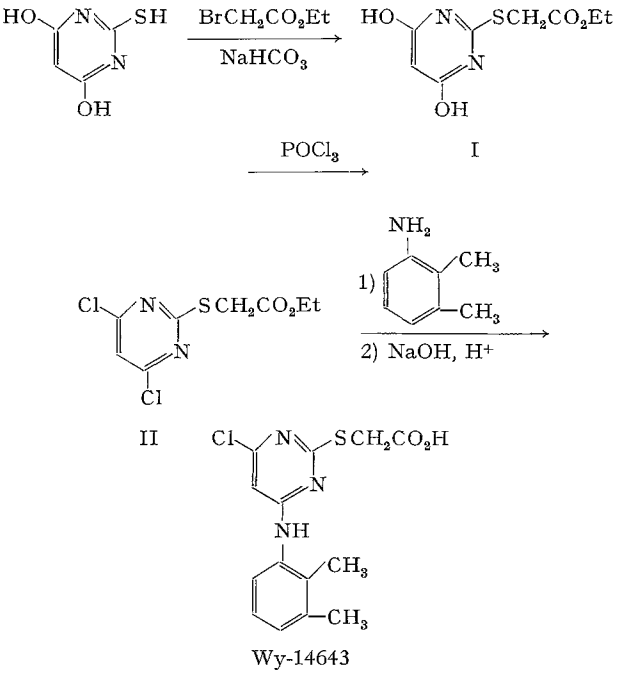
A Potent Antihypercholesterolemic Agent: [4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic Acid (Wy-14643)

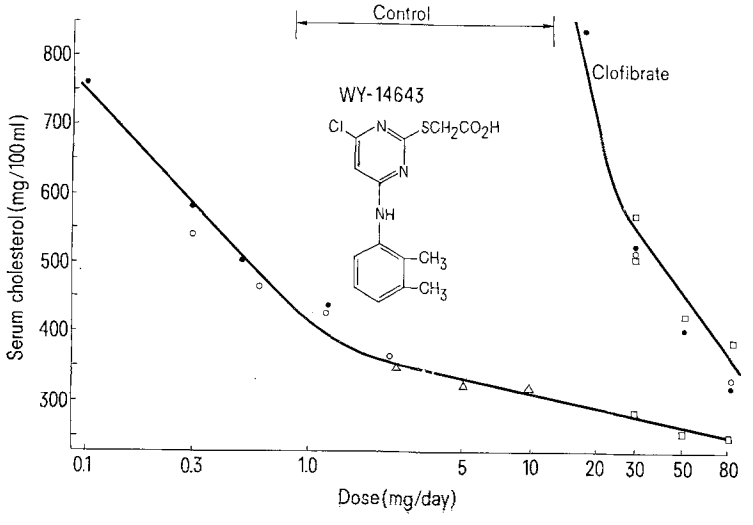
The search for more efficacious drugs to control hypercholesterolemia, a major risk factor in coronary heart disease, has been intensified over the past decade. One of the most widespread agents used in the clinical management of hyperlipidemia is ethyl *p*-chlorophenoxyisobutyrate (clofibrate)[1]. A number of 2-pyrimidinylthioacetic acid derivatives recently synthesized in this laboratory were compared with clofibrate for ability to lower the serum cholesterol in rats with hypercholesterolemia of dietary origin. The pertinent points of this assay procedure have previously been described by one of us[2]. Since these pyrimidine derivatives represent a novel class of hypocholesterolemic agents, it is the purpose of this communication to outline the synthesis and biological activity of one of the more potent and interesting members of the series, [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid (Wy-14643).

The scheme illustrates the route used to prepare the title compound. The alkylation of sodium 2-thiobarbiturate with an equivalent of ethyl bromoacetate in aqueous ethanol at 60° for 1.5 h gave a 72% yield of I: m.p. 194–197°/ethanol; analysis for $C_8H_{10}N_2O_4S$, Calc: C, 41.37; H, 4.38; N, 12.16. Found: C, 41.75; H, 4.46; N, 12.27.

Ethyl (4,6-dichloro-2-pyrimidinylthio)acetate (II) was obtained in 71% yield by treating I in boiling phosphorus oxychloride containing N,N-diethylaniline for 4 h. Removal of the phosphorus oxychloride and treatment of the residue with ice gave II: m.p. 61–62°/petroleum ether; analysis for $C_8H_8Cl_2N_2O_2S$, Calc: C, 35.97; H, 3.02; N, 10.48. Found: C, 35.95; H, 3.00; N, 10.30.

Treatment of II with an equivalent of 2,3-dimethylaniline and sodium carbonate in boiling ethanol for 4 h, followed by filtration, and addition of water to the filtrate gave 61% yield of ethyl [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetate: m.p. 87–91°/ethanol (95%); analysis for $C_{16}H_{18}ClN_3O_2S$, Calc: C, 54.62; H, 5.15; N, 11.94. Found: C, 54.80; H, 5.08; N, 12.00.





Effect of Wy-14643 and clofibrate on the serum cholesterol of hypercholesterolemic male rats (250–350 g body weight). The data from 4 bioassays are combined; values represented by a given symbol are from the same bioassay.