Inhibitive Effects of Structurally Modified Azasteroids and Related Nitrogen Containing Steroids on Insect Growth and Development¹

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ABSTRACT

A number of azasteroids and other nitrogen containing steroids with a modified nucleus or side chain were prepared and tested for their inhibitory effects on the growth and development of several species of insects. Structure-activity studies showed that compounds with a structurally related steroid nucleus and side chain were approximately equal in inhibitory activity for a particular species. The replacement of the tertiary amino group in the side chain of the 5 β -steroid with other nitrogen substituents, such as nitro, cyano, acetylamino, or a quaternary ammonium salt, resulted in a considerable loss of inhibitive activity in the tobacco hornworm or the yellowfever mosquito. However, certain modifications of the azasteroid nucleus resulted in compounds that still retained high biological activity. As a result, a compound was synthesized that lacked the A and B rings of the steroid nucleus and that inhibited insect growth, molting, and metamorphosis and the Δ^{24} -sterol reductase enzyme system of the tobacco hornworm.

INTRODUCTION

Previous studies of the structure-activity relationships of a number of azasteroids showed that certain 25-azasterols were more effective in inhibiting insect growth and development than either diazasterols or monoazasterols with a secondary nitrogen at the 23 or 24 position (1). As a result, we were able to design and synthesize a number of new 25-azasteroids that were considerably more active inhibitors for several species of insects (2). These azasteroids were also potent inhibitors of the Δ^{24} -sterol reductase enzyme, caused an accumulation of desmosterol, and blocked the conversion of plant sterols to cholesterol in certain insects. Subsequently, in an effort to determine whether the tertiary nitrogen was a prerequisite for maximum biological activity and to determine the minimal structural requirement for this activity, we synthesized a number of new nitrogen containing steroids with modified and/or shortened side chains and modified steroid nuclei. In the present paper, we report the synthesis and compare the inhibitory activities of these new compounds with those of our previous 3 most active 25-azasteroids on 4 species of insects. The IUPAC equivalent names used throughout this paper are listed in Figure 1.

EXPERIMENTAL PROCEDURES

Biological Test Systems

The larval test systems for the yellowfever mosquito, Aedes aegypti (L.), the confused flour beetle, Tribolium confusum Jacquelin duVal, and the house fly, Musca domestica L., were those previously used to assess the inhibitive effects of ecdysone and synthetic analogs on growth and metamorphosis (3). The larval test system for the tobacco hornworm, Manduca sexta (L.), was as previously described for testing azasteroid inhibitors (1).

Instrumentation

Melting points were observed on a Kofler block, and infrared (IR) spectra were obtained with a Perkin-Elmer model 221 prism-grating spectrophotometer. Gas liquid chromatographic (GLC) analyses were made on a Barber-Colman model 10 chromatograph equipped with a beta ionization detector cell. GLC systems were 0.75% SE-30 and 1% OV-17 coated on Gas-Chrom P and the columns temperatures were 236 and 230 C, respectively. NMR spectra were recorded at 60 Mc with a Varian A-60A NMR spectrometer with deuterated chloroform as the solvent and trimethyl silyl (TMS) as an internal nuclear magnetic resonance (NMR) standard. The mass spectra were obtained by using an LKB model 9000 gas chromatograph mass spectrometer (LKB Produckter AB, Stockholm, Sweden). Samples were introduced directly into the ionization chamber (ionization energy 70 ev.) except for compound XLI which was introduced through the gas chromatography (GC) system.

Materials and Chemical Synthesis

Basic and neutral alumina (Woelm) were obtained from Waters Associates Inc. (Framingham, MA), and the required amount of water

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 $XVI = 3\beta$ -Methoxy-chol-5-en-24-dimethylamine XVII = 3\beta-Methoxy-24,23-dinorchol-5-en-22-dimethylamine XVIII = 3β-Trimethylsiloxy-chol-5-en-24-dimethylamine XIX = 5α -Cholan-24-dimethylamine XX = 24,23-Dinor-5 α -cholan-22-dimethylamine $XXI = 5\alpha$ -Cholan-24-methylethylamine XXII = 5β -Chol-2-en-24-dimethylamine and 5β-Chol-3-en-24-dimethylamine XXIII = 5β -Chola-2,7,11-trien-24-dimethylamine XXIV = 2,3-Seco-5 β -cholan-24-dimethylamine XXV = 3,4-Seco-5 β -cholan-24-dimethylamine XXVI = 2ξ , 3ξ -Dichloro- 5β -cholan-24-dimethylamine XXVII = 3α -Hydroxy- 5β -cholan-24-dimethylamine XXVIII = 3α -Trimethylsiloxy-5 β -cholan-24-dimethylamine $XXIX = 5\beta$ -Cholan-24-dimethylamine XXX = 24-Nor-5 β -cholan-23-dimethylamine XXXI = 24,23-Dinor-5 β -cholan-22-dimethylamine XXXII = 5β -Cholan-24-trimethylammonium iodide XXXIII = 24,23-Dinor-5\beta-cholan-22-aminoethanol-22-p-toluenesulfonate $XXXIV = 5\beta$ -Cholan-24-amine XXXV = 24-Nitro-5 β -cholane XXXVI = 5β -Cholan-24-acetamide XXXVII = 24-Dimethylureido-5 β -cholane XXXVIII = 24-Cyano-5 β -cholane XXXIX = 24-Nor-23-cyano-5 β -cholane XL = 24,23-Dinor-5 β -cholan-22-diethylphosphate XLI = N, N, δ -7a-Tetramethyloctahydro-1-H-indene-1-butanamine.

FIG. 1. IUPAC equivalent names.

was added to make activity Grade II alumina. Thin layer chromatographic (TLC) analyses were made on Quanta/gram precoated silica gel plates (Quantum Industries, Hanover, NJ). Cholic acid was purchased from Nutritional Biochemicals Corp. (Cleveland, OH), and 5 β -cholanic acid was readily prepared via oxidation of cholic acid methyl ester to the triketone, dehydrocholic acid methyl ester, and its subsequent reduction and saponification by the Wolff-Kishner reaction. Lithocholic acid (3 α -hydroxy-5 β -cholanic acid) was prepared from methyl cholate by the methods of Sarel and Yanuka (4).

The intermediate acid required for the preparation of compound XLI was prepared by ozonolysis of vitamin D_2 (5,6) and chromic acid oxidation of the keto-aldehyde in acetone with an 8N solution of chromic acid in dilute sulfuric acid (7). A Wolff-Kishner reduction of the resulting keto-acid gave the intermediate β -7*a*-dimethyloctahydro-1*H*-indene-1-acetic acid. The reaction of this acid with thionyl chloride gave the acid chloride, which immediately was allowed to react with diazomethane to give the diazoketone. An Arndt-Eistert rearrangement of the diazoketone by the modified procedure of Wilds and Meader (8) gave the benzyl ester of an acid with its chain length increased by one carbon from that of the initial acid. An alkaline saponification of this ester and a repeat of Wilds procedure gave the acid of the desired chain length for preparing the amine XLI in a 30% overall purified yield from vitamin D_2 .

The new azasteroids were all prepared in 60-80% yield according to the general method of synthesis via reaction of the appropriate steroidal acid with thionyl chloride to give the steroidal acid chloride and its reaction with dimethylamine, methylethylamine, or ammonia to give the amide and its subsequent reduction with lithium aluminum hydride in tetrahydrofuran to the amine (9). The dichloroazasteroid XXVI was prepared in 50% yield via chlorination of 5 β -chol-2-en-24-dimethylamine in carbon tetrachloride at 5 C.

The quaternary ammonium salt XXXII was prepared in 90% yield by the reaction of the tertiary amine XXIX and methyl iodide in acetone: acetonitrile (3:1) in a sealed tube overnight at 70 C. The ammonium tosyl salt XXXIII was readily prepared in 95% yield by heating at 75 C for 1 hr a mixture of 2-dimethylaminoethanol and the 22-tosylate of 24,23dinor-5 β -cholan-22-ol, prepared from lithium aluminum hydride reduction of the methyl ester of XVb (Scheme II).

The oxidation of the primary amine XXXIV with *m*-chloroperbenzoic acid gave the nitro derivative XXXV in 45% purified yield. Compound XXXVI was prepared in nearly quantitative yield by the reaction of XXXIV in pyridine at room temperature overnight with acetic anhydride. The reaction of XXXIV with dimethyl carbamyl chloride in xylene containing 6 equivalents of triethylamine at reflux temperature for 2 hr gave XXXVII in 77% yield. The reaction of the 24-tosylate of 5 β -cholan24-ol with sodium cyanide in a solution of dimethylsulfoxide:benzene (3:1) at room temperature overnight gave the cyano compound XXXVIII in a 72% purified yield. A similar reaction of the 23-tosylate of 24-nor-5 β -cholan-23-ol with sodium cyanide gave compound XXXIX. The reaction of 24-nor-5 β -cholan-23-ol at reflux temperature for 7 hr with diethyl chlorophosphite in benzene containing 2 equivalents of triethylamine gave the diethylphosphate ester XL in a 65% yield. The TMS derivatives XVIII and XXVIII were prepared in nearly quantitative yield by the reaction of 25-azacholesterol and XXVII, respectively, in pyridine at 70 C overnight with N,N-bis(trimethylsilyl) acetamide.

When necessary, the compounds were purified by column chromatography. The structures of the intermediates and final products (Fig. 2) were confirmed by IR, NMR, and mass spectroscopy. Purity of the final products as determined by GLC and TLC was > 98%.

Mass spectra of all compounds shown in Figure 2 gave strong M⁺ and M-15 peaks (Table I) except for the quaternary ammonium salt XXXII and compound XXXIII. Spectra of all the N,N-dimethylamine compounds showed base peaks at m/e 58 that resulted from the simple fission of the carbon-carbon bond adjacent to the nitrogen atom (α -cleavage). A base peak at m/e 72 in the spectrum of compound XXI indicated a similar cleavage for this compound. The base peak for the primary amine XXXIV occurred at m/e 84, which was the side chain fragment resulting from fission of the 17-20 bond. In the spectra of compounds XXXV-XXXIX, the base peaks occurred at m/e 217, which was the fragment of the steroid nucleus resulting from fission of the 13-17 and 14-15 bonds of the D-ring. In the spectrum of the quaternary ammonium salt XXXII, the base peak occurred at m/e 142 which was the methyl iodide fragment; in the spectrum of XXXIII the base peak occurred at m/e 300, the 24,23-dinor-5 β -cholene fragment. Additional physical properties of compounds of Figure 2 are given in Table 1.

General Procedure for the Preparation of Intermediates in Schemes I and II

Methyl 5 β -chol-2-enoate (IIa, Scheme I) and methyl 5 β -chol-3-enoate (IIIa, Scheme I). A mixture of 24.3 g methyl 3 α -tosyl-5 β -cholanoate (Ib) and 12.15 g each of lithium carbonate and lithium bromide was refluxed for 1 hr. The solution was filtered while hot, and the filtrate was cooled and poured into ice and water. The semicrystalline material was collected and then dried under vacuum to give 15 g of a ca. 1:1



FIG. 2. Structures of nitrogen containing steroids.

mixture of IIa and IIIa as indicated by GLC analyses. The crude mixture was dissolved in hexane and filtered through 100 g hexanewashed alumina. The first 500 ml of hexane eluted 5.7 g of material, and the next 250 ml of hexane:benzene (1:1) eluted an additional 5.7 g of material. Approximately 5.7 g of the material from the hexane fraction dissolved in 30 ml hexane was placed on a 400-g column of Unisil impregnated with 20% silver nitrate in a column 5 x 37 cm tapered to 3 x 43 cm. The column was developed by gradient elution with 1 liter of benzene added dropwise into a 1-liter mixing flask filled with hexane, which was added directly to the column. Once the benzene had been added to the mixing flask, 500 ml of a mixture of benzene: hexane (6:1) also was added dropwise to the mixing flask. Approximately 850 ml of solvent was passed through the column; then 80 20-ml fractions were collected. When the fractions were monitored by GLC on an OV-17 column, fractions 30-40 contained the Δ^3 -compound with > 98% purity, and fractions 55-78 contained the Δ^2 -compound with > 95% purity. Intermediate fractions containing varying mixtures of the 2 compounds were combined on the basis of degrees of purity for rechromatography. From 3 chromatographic fractionations, we obtained 3.0 g of > 98% pure Δ^3 -compound (IIIa, Scheme I), mp 74-76 C, NMR, δ 0.67 (s, 3, 18-methyl), 0.99 (s, 3, 19-methyl), 0.93 (d, 3, J = 6 Hz, 21-methyl), 3.68 (s, 3, COOCH₃), multiplet at 5.22-5.78 (olefinic protons); and 4.0 g of > 95% pure Δ^2 -compound (IIa, Scheme I), mp 70-71 C, NMR, δ 0.67 (s, 3, 18-methyl), 0.97 (s, 3, 19-methyl), 0.91 (d, 3, J = 5 Hz, 21-methyl), 3.68 (s, 3, COOCH₃),

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Physical Properties of Nitrogen Containing Steroids

			Nuclear n	lagnetic resonanc	ie (δ)		Mass spectral da	ta
	Malting noint	N	Aethyl resonar	ıces	N N-Dimethul		M ⁺ - firet	
Compound ^a	(C)	1.8-H	19-H	21-Н ^р	resonances	Base peak	loss	Molecular ion
XVI	133-134	0.68	1.00	0.93	2.22(s) ^c	58	386	401
XVII	115-117	0.73	1.03	1.02	2.22(s)	58	358	373
XVIII	102-104	0.68	1.00	0.91	2.22(s)	58	444	459
XIX	87-88	0.65	0.78	0.95	2.22(s)	58	358	373
XX	128-130	0.70	0.80	0.93	2.20(s)	58	330	345
XXI	78-79	0.66	0.78	0.95	2.22(s)d	72	372	387
XXII	235-240 ^e	0.67	0.97	0.92	2.24(s)	58	356	371
XXIII	215-218 ^e	0.63	0.84	0.95	2.24(s)	58	352	367
XXIV	67-68	0.66	0.92	0.91	2.22(s)	58	360	375
XXV	I	0.66	0.93	0.92	2.22(s)	58	360	375
ΙΛΧΧ	,	0.67	1.00	0.92	2.28(s)	58	426	441
ΙΙΛΧΧ	109-112	0.65	0.92	0.93	2.22(s)	58	374	389
XXVIII		0.64	0.90	0.92	2.22(s)	58	446	461
XIXX	63-65	0.66	0.94	0.93	2.22(s)	58	358	373
XXX	80-81	0.66	0.92	0.93	2.22(s)	58	344	359
XXXI	67-68	0.68	0.93	0.93	2.22(s)	58	330	345
XXXII	268-269	0.65	0.92	0.98	3.52(s)	142	373	5 44
XXXIII	206-208	0.65	0.91	1.12	3.22(s)	300	472	Į.
XXXIV	225-231 ^e	0.67	0.94	0.95	•	84 ,	330	345
XXXV	I	0.63	0.92	0.93		217	360	375
ΧΧΧΝ	108-109	0.67	0.93	0.91	1.978	217	372	387
ΧΧΧΝΙΙ	156-157	0.65	0.93	0.92	2.91	217	401	416
ΙΙΊΛΧΧΧ	108-112	0.66	0.93	0.94		217	340	355
XXXIX	126-128	0.68	0.93	0.92	ı	217	326	341
XL	24-26	0.63	0.91	0.95	ı	155	453	468
XLI	1	0.87 ⁿ	•	0.931	2.21(s)	58	236	251

^aSee Figure 1 for IUPAC equivalent names. ^bFor the 21-methyl resonance, J = 4-6 cps.

c(s) = singlet.

dThe N-methylene of the N-ethyl appears as a multiplet at δ 2.0-2.6.

^eMp of the amine hydrogen chloride.

fSpectrum showed no molecular ion. gN-acetyl resonance.

ⁱThe 7a-methyl resonance of compound XLJ. hThe 6-methyl resonance of compound XL1.

multiplet at 5.60 (olefinic protons).

Methyl 2,3-bisethylenedithioketal-2,3-seco-5\beta-cholanoate (V, Scheme I) and methyl 3,4bisethylenedithioketal-3, 4-seco-5 β -cholanoate (VIII, Scheme I) via the dialdehydes VI and VII (Scheme I), respectively. A solution of 2.0 g of IIa or IIIa (Scheme I) in 40 ml methylene chloride at -70 C was treated with ozone until a slight excess was present (ca. 3 hr). To the cold solution, 2.0 g zinc dust and 10 ml acetic acid were added, and the stirred mixture was allowed to come to room temperature. After stirring for 1 hr, the solution was filtered, and the filtrate was concentrated to a small volume, diluted with water, and extracted with hexane. The hexane solution was washed first with 5% sodium bicarbonate solution and then with water and dried over anhydrous sodium sulfate. Removal of the solvent under vacuum gave 2.1 g of the oil dialdehyde IV or VII (Scheme I). To 2.0 g crude dialdehyde (IV, Scheme I) in 1 ml ether and 1 ml ethanedithiol at 5 C, 1.1 ml boron trifluoride etherate was added. After 10 min of stirring the mixture with a glass rod, a thick paste was formed. The paste was triturated with hexane and filtered, and the precipitate was washed with 70% aqueous methanol. The yield of the bisethylenedithioketal derivative V (Scheme I) was 1.8 g, mp 156-158 C, NMR, δ 0.64 (s, 3, 18-methyl), 1.12 (s, 3, 19-methyl), 0.89 (d, 3, J = 4 Hz, 21-methyl), 3.28 [m, 10, -CH(-S-CH₂CH₂S-)] 3.67 (s, 3, COOCH₃).

The crude dialdehyde VII (Scheme I), under similar experimental conditions, yielded 1.75 g of 3,4-bisethylenedithioketal derivative VIII (Scheme I), mp 121-123 C, NMR, δ 0.65 (s, 3, 18-methyl), 0.98 (s, 3, 19-methyl), 0.89 (d, 3, J = 5 Hz, 21-methyl), 3.20 [m, 10, -CH(-SCH₂CH₂S-)], 3.67 (s, 3, COOCH₃).

Methyl 2, 3-seco-5 β -cholanoate (VIa, Scheme I) and Methyl 3,4-seco- 5β -cholanoate (IXa, Scheme I). A mixture of 1.7 g bisethylenedithioketal (V, Scheme I), 40 ml dry dioxane, and 1.5 teaspoons (ca. 4.5 g) of Raney nickel catalyst (W-2) was refluxed overnight. The solution was mixed with a small quantity of Celite, filtered, and concentrated to dryness under vacuum to give 1.2 g residue. The residue was chromatographed over 30 g hexane-washed neutral alumina (activity grade II), and 100-ml fractions were collected, 2 of hexane, and 3 of hexane:benzene (1:1). On the basis of TLC analyses, the first 2 fractions of hexane:benzene mixture were combined to give, after crystallization from acetonitrile, 1.0 g VIa (Scheme I), mp 38-40 C, NMR, δ 0.66 (s, 3, 18-methyl), 0.92 (s, 3, 19-methyl), 0.89 (d, 3, J = 5 Hz, 21-methyl), 3.68 (s, 3, COOCH₃).



Scheme I. Synthesis of intermediates for preparation of compounds in Figure 2.



Scheme II. Synthesis of intermediates for preparation of compounds in Figure 2.

The Raney nickel catalytic reduction of 2.6 g 3,4-bisethylenedithioketal VIII (Scheme I) and work-up as in the reduction of V (Scheme I) gave 1.6 g noncrystalline IXa (Scheme I), NMR, δ 0.66 (s, 3, 18-methyl), 0.89 (d, 3, J = 5 Hz, 21-methyl), 3.68 (s, 3, COOCH₃).

Methyl 5 β -chola-2,7,11-trienoate (XIa, Scheme II) and its carboxylic acid (XIb, Scheme II). A mixture of 7.0 g methyl 3α , 7α , 12α -trimethylsulfonyl- 5β -cholanoate (X, Scheme I), prepared from the mesylation of cholic acid methyl ester, and 2.5 g each of lithium carbonate and lithium bromide and 60 ml dimethylformamide were refluxed for 30 min. The solution was filtered while hot; the filtrate was cooled and then poured into ice and water; and the precipitate was collected. Crude precipitate of methyl cholatrienoate (XIa, Scheme I) was saponified with 5% potassium hydroxide in 90% ethanol; the solution was diluted with water and acidified with 6N hydrochloric acid; and the precipitate was collected. Recrystallization twice from acetone: methanol gave 4.1 g XIb (Scheme II), mp 170-172 C. A sample of acid was converted to the methyl ester (XIa, Scheme II) with diazomethane. The GLC analyses of this sample of the methyl ester on an SE-30 column showed only one peak. The GLC

analyses of the final product XXIII also showed only one peak, which suggested that primarily the methyl 5β -chola-2,7,11-trienoate was formed during the demesylation.

24-Nor-5β-cholanic acid (XVa, Scheme II) and 24.23-dinor-5 β -cholanic acid (XVb, Scheme II). The methyl 5 β -cholanoate (XIIa, Scheme II) or the methyl 24-nor-5 β -cholanoate (XIIb, Scheme II) in benzene was added to a refluxing solution of 4 equivalents of phenylmagnesium bromide in ether. After the reaction mixture had refluxed for 3 hr, an additional quantity of benzene was added; the ether was distilled off; and the mixture then was refluxed overnight. The benzene solution was poured into ice and water, and the mixture was acidified with a dilute solution of hydrochloric acid. The mixture then was extracted with benzene, and the benzene extract was washed with water, dried over sodium sulfate, and concentrated to dryness under vacuum to give the cholanyldiphenyl carbinol (XIIIa, Scheme II) or the 24-norcholanyldiphenyl carbinol (XIIIb, Scheme II). The diphenyl carbinol derivatives (15 g) in 150 ml dioxane containing 15 ml sulfuric acid, by stirring overnight at room temperature, were converted to the diphenylethylene derivatives XIVa (Scheme II), mp 117-118 C, and XIVb (Scheme II), as an oil. Ozonization of XIVa (Scheme II) in methylene chloride at -80 C and oxidation of the resultant crude aldehyde mixture with chromic acid solution in acetone (7) gave, in nearly quantitative yield, the acid XVa (Scheme II), mp 175-177 C (lit.(10), mp 177 C). A similar sequence of reactions with XIVb (Scheme II) gave the dinoracid XVb (Scheme II), mp 209-212 C (lit.(10), mp 214 C).

RESULTS AND DISCUSSION

The inhibitive ranges of concentrations of these newly prepared compounds in the yellowfever mosquito and the tobacco hornworm are presented in Table II. Compounds XVI, XIX, and XXIX, which were our most active azasteroids in previous tests (2), are included for comparison. Because the confused flour beetle and the house fly were not particularly affected by the majority of these new compounds, the results of the biological tests with these 2 insects will be summarized briefly.

In the house fly test system, only 6 of the compounds listed in Table II (XVI, XVII, XXIV, XXV, XXXI, and XXXIV) inhibited growth and development of or killed 75% of the test insects at a dietary concentration of 150-375 ppm. However, several of the other azasteroids at concentrations within this range did cause the characteristic effects previously

observed for azasteroid inhibitors (2). Of the 6 active compounds, only compound XVII has the steroid nucleus similar to XVI, our most active azasteroid in this insect, though it differed in its side chain by having 2 less carbon atoms. The other 4 compounds with activity in this insect all have the A/B ring *cis* configuration; yet, they differ from each other in several respects including chain length, opened ring A, or the possession of a primary amino group.

Of the more than 80 azasteroids tested on the confused flour beetle, only compounds XVI and XVII, which possessed a Δ^5 -bond and a 3β -methoxy group, were active at a dietary concentration of < 100 ppm. Except for compounds XXVI and XXXVII, that were active in this insect at concentrations between 500-1000 ppm, all other compounds listed in Table II were inactive even at concentrations of 1000 ppm. The results suggest that an azasteroid such as XVI with a steroid nucleus without a functional group other than a Δ^5 -bond could be quite active in the confused flour beetle.

Although the majority of the new nitrogen containing steroids were not very active in either the confused flour beetle or the house fly, a number of these compounds were active at < 1 ppm in the yellowfever mosquito and the tobacco hornworm. The results of tests of the yellowfever mosquito with Δ^5 -steroids showed that compounds XVI and XVII were equally active, but the TMS-derivative XVIII was only ca. one-tenth as inhibitive as XVI. Of the azasteroids with the A/B ring trans configuration, compound XX, with the shortened side chain, had a greater inhibitive effect than XIX. However, the substitution of an N-ethyl group for an N-methyl, as in compound XXI, resulted in no change in biological activity from that of XIX.

When we compare the inhibitive effects of the 17 compounds with an A/B ring cis configuration in the mosquito with the activity of compound XXIX, only compounds XXX and XXXI, which have a shorter side chain than XXIX, and compounds XXXII and XXXIV were more active. Interestingly, compound XXXIV, a steroidal primary amine, was ca. 4 times more active in the yellowfever mosquito than the steroidal tertiary amine XXIX. The A/B ring cis steroids XXXV-XXXIX, which are without a tertiary amino group, were inactive at a concentration of 10 ppm except for the acetyl derivative XXXVI, which was active at concentrations of 5-10 ppm in this insect. The seco compounds XXIV and XXV and compound XXII, which consists of a 1:1 mixture of the 2and 3-ene compounds, were equal in activity to XXIX in the yellowfever mosquito larvae. The

TABLE II

Compound ^a	Yellowfever mosquito (ppm)	Tobacco hornworm (ppm)
Δ^5 -Azasteroids		
XVI	0.50 - 1.00	0.25 - 0.50
XVII	0.50 - 1.00	4.00 - 8.00
XVIII	5.00 -10.00	33 -65
5a-Azasteroids		
XIX	0.50 - 1.00	0.50 - 0.75
XX	0.25 - 0.50	4.00 - 8.00
XXI	0.50 - 1.00	0.50 - 0.75
Mono- and triunsaturated 56-azasteroids		
XXII	0.50 - 1.00	0.10 - 0.25
XXIII	1.00 - 2.50	0.10 - 0.25
Seco-5β-azasteroids		
XXIV	0.50 - 1.00	0.25 - 0.50
XXV	0.50 - 1.00	0.25 - 0.50
5β-Azasteroids		
XXVI	2.50 - 5.00	0.50 - 1.00
XXVII	2.50 - 5.00	1.00 -10.0
XXVIII	>10	<16
XXIX	0.50 - 1.00	0.10 - 0.25
XXX	0.25 - 0.50	0.25 - 0.50
XXXI	0.25 - 0.50	30
XXXII	0.25 - 0.50	>1 30
5β-Steroids with other nitrogen substituents		
XXXIII	2.50 - 5.00	>1 30
XXXIV	0.10 - 0.25	16 - 33
XXXV	>10	>65
XXXVI	5.00 -10.0	>1 30
XXXVII	>10	>260
XXXVIII	>10	>1 30
XXXIX	>10	>1 30
5β-Steroid with phosphorus substituent		
XL	>10	>260
Nonsteroidal amine		
XLI	5.0 - 10.0	130-260

Range of Concentrations of Nitrogen Containing Steroids in Larval Diet or Medium Required to Kill or Inhibit Development in 75% of the Test Insects

^aSee Figure 1 for IUPAC equivalent names.

dichloro derivative XXVI was active at concentrations of 2.5-5.0 ppm in this insect.

Of the compounds listed in Table II, only compounds XXII and XXIII, which have the A/B ring *cis* configuration, as does the previously most active azasteroid XXIX (2), were equal in activity to XXIX in the tobacco hornworm. Compound XXX, which has one carbon less in its side chain than XXIX, exhibited slightly less biological activity than XXIX; compound XXXI with 2 carbons less in its side chain than XXIX showed far less inhibitive activity. The presence of a nitrogen substituent other than the tertiary amino group in the side chain of the A/B ring *cis* steroids XXXII-XXXIX also resulted in a considerable loss of biological activity in the tobacco hornworm. The Δ^5 -azasteroid XVII, with a side chain containing 2 less carbon atoms than XVI, and the TMS derivative XVIII were far less inhibitive than the 3-methoxy- Δ^5 -azasteroid (XVI) in the tobacco hornworm. Additionally, the A/B ring *trans* azasteroid XX, which has a shorter side chain than XIX, showed far less biological activity than XIX in this insect. However, the replacement of an N-methyl group with an Nethyl as in XXI resulted in no change in activity in the tobacco hornworm. The phosphate ester XL was without inhibitive activity in all test systems at the highest dietary concentration examined.

In this comparative study, none of the new nitrogen containing steroids was appreciably more inhibitive than the most active azasteroids

tested previously (2). However, the results indicated that a prerequisite for an azasteroid that will inhibit insect growth and development was a side chain with a primary, secondary, or tertiary amino group, and in most instances preferably a tertiary amino group. Thus, to determine the minimal structural requirement for this type of inhibition of insect growth and development, we synthesized compound XLI, which lacked the A and B rings of the steroid nucleus. This compound inhibited growth and development in both the yellowfever mosquito and the tobacco hornworm though it was not as potent as the more active azasteroids (Table II). Also like certain azasteroids, it blocked the Δ^{24} -sterol reductase enzyme of the tobacco hornworm. At 130 ppm in the diet, it caused reduction of the cholesterol level to < 5% of the total tissue sterol as opposed to the 80-85% found in control insects, and increased the desmosterol content from the normal range of 1.0-1.5% to ca. 50% of the total sterol present in the tobacco hornworm.

Our previous results with azasteroid inhibitors (2) demonstrated the feasibility of disrupting the hormone mediated processes of insects with relatively simple nonhormonal compounds that apparently interfere with hormone biosynthesis and metabolism. As a result of the present study, we now have synthesized simple nonsteroidal amines, some of which proved to be inhibitors of insect growth, molting, and metamorphosis (11). These nonsteroidal compounds should permit us to expand the type of chemicals that block the hormone regulated processes of growth, molting, and metamorphosis in insects, and, thus, may lead to the development of new types of safe, selective insect control chemicals.

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