

Ecdysteroid analogs based on steroidal sapogenins I. Synthesis of bromo-derivatives from diosgenin. Preliminary study of their biological activity

Armando Zaldo Castro, Juan Enrique Tacoronte, Francisco Coll Manchado, Lucita Aguilera de la Paz* and María Teresa Cabrera.

University of Havana, Faculty of Chemistry, Dept. Organic Chemistry, Laboratory of Natural Products, Zapata and G. Ciudad de La Habana, Postal Code 10400, Cuba. *Department Control de Pest Carriers, Institute of Tropical Medicine "Pedro Kouri", Autopista del Mediodía km 6½, La Lisa, Ciudad de La Habana, Cuba.

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Key words: ecdysteroids, diosgenin epoxydation, bromination, biological activity.

RESUMEN. Los ecdiesteroides constituyen una larga familia de esteroides con más de 100 compuestos, distribuidos en los reinos vegetal y animal, donde desempeñan un papel importante en los procesos de desarrollo, metamorfosis y reproducción. En las plantas actúan como protectores contra insectos fitófagos. En esta comunicación se describe la síntesis de bromoderivados esteroidales como potenciales agentes insecticidas e intermediarios avanzados para la obtención de análogos de ecdiesteroides a partir de substratos espirostánicos de procedencia natural y comercialmente asequibles tales como la diosgenina, y la elucidación estructural de los compuestos obtenidos mediante espectroscopia infrarroja, FTIR, y resonancia magnética nuclear RMN ^1H - ^{13}C . Durante los procesos de obtención de más de 10 intermediarios fueron utilizadas y optimizadas reacciones de epoxidación y su apertura oxidativa, técnicas de hidroxilación *cis* a dobles enlaces y halogenación selectiva a cetonas. Los estudios preliminares para la detección de actividad biológica muestran que estos derivados bromados constituyen potenciales insecticidas para el control de plagas de *Blatella germanica* (cucaracha alemana) en comunidades humanas.

ABSTRACT. Ecdysteroids represent a large family of steroids comprising more than 100 compounds. They have been found in both invertebrates and plant kingdom where they play an important role in some facets of development, metamorphosis and reproduction. In plants, they provide a potential protection against phytophagous predators. The aim of the undertaken report is the synthesis and structural elucidation, through NMR (^1H - ^{13}C) and FTIR techniques, of advanced intermediates synthesized from naturally and commercially available steroidal sapogenin diosgenin. Some classic reactions and optimized variants were used for obtaining more than 10 intermediates: epoxydations, and oxidative cleavage of oxyranic rings, *cis*-hydroxylation to double bonds and α -halogenation to carbonyl compounds. The biological activity of several obtained intermediates was researched in order to establish the real potential of these derivatives as insecticides. The results suggest that this kind of bromine derivatives could be used as genetic modulator for controlling *Blatella germanica*, a typical cockroach pest in human communities.

INTRODUCTION

Ecdysteroids represent a large family of polyhydroxylated steroids widely distributed in several groups of invertebrates (zooecdysteroids) and species of the plant kingdom (phytoecdysteroids).¹⁻⁵ They were first isolated in insects,⁶ and their isolation is extended in crustacean and other arthropods,^{7,8} mollusks,^{9,10} annelids¹¹⁻¹⁴ and coelenterates.¹⁵

In insects, those isolated substances at very small concentrations, control the principal biochemical and metabolic events of these arthropods' life cycle, such as genetic expression processes, molting and metamorphosis, gonads maturation, reproduction and excretion of determinate protohormones related with chrono-circadian and defensive cycles.¹⁶⁻¹⁹ In plants, they have a potential defensive action against the phytophagous species of insects and acari.²⁰⁻²⁶

The discovery of the hormonal meaning of 6-ketosteroids in crustaceans and insects²⁷ has generated a change in the philosophy of the rational pest control, when it increases the possibilities of use of ecdysteroids analogs in the techniques of molting and metamorphosis of insects for an effective population control and ecologically sustainable.²⁸⁻³¹

The purpose of this paper is to report the use, as starting substrate, of diosgenin, a steroidal sapogenin commercially available in Cuba, for the synthesis of ecdysteroids bromo-derivatives potentially bioactive in the fight against *Blattella germanica* species, a widely extended pest in human communities.

MATERIALS AND METHODS

The determination of the fusion temperatures of the synthesized compounds was performed by using an oven with Electrothermal 9100 capillary. Those temperatures were not corrected.

The FTIR spectra were recorded in a Philips Analytical PU 9600 FTIR spectrophotometer in the 450-4 500 cm^{-1} interval in KBr pellets at room temperature.

The Magnetic Nuclear Resonance spectra (MNR ^1H - ^{13}C) were performed in a Bruker ACF-250 equipment. The samples were solved in deuterated chloroform, by using TMS as internal reference.^{32,33}

Silicagel 60 Merk (70-230 mesh ASTM) was used for separating in chromatographic columns. The synthesis processes described were controlled by thin-layer chromatography by using silica pre-elaborated chromatographic plates of 0.25 mm thickness. As developer, a vainilline solution 1 % in 50 % perchloric acid was used.

Reagents from Merk, Panreac and BDH were used. The solvents, with technical quality, were dried and distilled before use. The diosgenin was granted by the Center of Studies of Natural Products, Faculty of Chemistry, University of Havana.

The separation and purification process of the synthesized compounds by column chromatography (CC) was developed by using as solvent a mixture of n-hexane/ethyl acetate (v/v) varying according to the derivatives.

For the IV compound: hexane-ethyl acetate at a rate of 4:1 (v/v).

For the VI, VII, VIII, XI compounds: hexane-ethyl acetate at a rate of 8:1 (v/v).

For the IX compound: hexane-ethyl acetate at a rate of 1:4 (v/v).

EXPERIMENTAL PART

(25R)-5-spirostan-3 β -ol, 3-acetate (I)

Five grams of diosgenin are solved in 100 mL pyridine and 50 mL acetic anhydride. The mixture is settled for 24 h and poured on ice-water. The obtained precipitate is

filtered and washed with diluted chlorhydric acid (HCl) and water. An amount of 5.2 g I are obtained. T.f. 195-196 °C. FTIR (cm^{-1} , v, KBr) 2 975, 1 735 (C=O, acetate), 1 457, 1 393, 1 050, 990, 958, 891 (spirocetalic system).

(25R)-5,6-epoxide-spirostan-3 β -ol, 3-acetate (II)

Three grams (17.53 mmol) meta-chloroperbenzoic acid (A-MCPB) are added to a solution of I (5 g; 10.96 mmol) in 20 mL CHCl_3 at room temperature. The reacting mixture is stirred for 30 min, diluted with 25 mL chloroform and successively washed with Na_2SO_3 , Na_2CO_3 aqueous solutions and finally with water. The organic phase is dried and the solvent is vacuum-evaporated. Yield: 4.39 g. T.f. 207-209 °C. FTIR (cm^{-1} , v, KBr) 2 944, 1 731, 1 456, 1 304, 1 243, 1 040, 974, 897. MNR- ^1H (δ , ppm) 0.68 (s, CH_3 -18); 0.78 (d, CH_3 -27); 0.9 (d, CH_3 -21); 1.06 (s, CH_3 -19); 1.98 y 1.99 (s, CH_3 -acetate); 2.97 y 3.07 (d, H-6); 3.39 (m, 2H-26); 4.35 (m, H-16); 4.71 y 4.93 (H-3).

(25R)-spirostan-3 β -5 α -dihydroxi-6-one-3-acetate (III)

To a 4 g (8.47 mmol) solution of II in 30 mL acetone, 2.3 g (23 mmol) CrO_3 solved in water (5 mL) were added in such a way that the reacting mixture is kept in conditions of smooth reflux. When the reaction is ended, the mixture is poured on iced water. The solid precipitating is filtered, washed with water and recrystallized of methanol. Yield: 3.71 g. T.f. 280-283 °C. FTIR (cm^{-1} , v, KBr) 3 386, 2 951, 1 709, 1 453, 1 241, 1 049, 977, 898. MNR- ^1H (δ , ppm) 0.71 (s, CH_3 -18); d, CH_3 -27); 0.81 (s, CH_3 -19); 0.82 (s, CH_3 -21); 2.01 (s, CH_3 -acetate); 3.49 (m, 2H-26); 3.58 (s, OH); 4.41 (m, H-16); 5.01 (m, H-3) are obtained.

(25R)-spirostan-7 α ,23 α ,23 β -tri-bromo-3 β , 5 α -dihydroxi-3-acetate (IV)

A solution of III (1.5 g; 3.08 mmol) in 20 mL CHCl_3 is treated with 8.5 mL of a Br_2 solution (2.5 mL; 7.79 g; 15.56 mmol) and 6 mL de AcOH in CHCl_3 (90 mL). The reacting mixture is settled for 2 h at room temperature. After this period, the mixture is poured on a Na_2SO_3 aqueous solution 10 %, is extracted with benzene and this extract is washed with a NaHCO_3 saturated aqueous solution and then with water. The extract is dried and the solvent is vacuum-evaporated. The solid is

purified by column chromatography and 1.99 g of the product is obtained. T.f. 189 °C. FTIR (cm^{-1} , v, KBr) 3 416, 1 713, 1 264, 1 033, 969. MNR- ^1H (δ , ppm) 0.82 (s, CH_3 -18); 1.02 (s, CH_3 -19); 1.23 (d, CH_3 -27); 1.98 (s, CH_3 -acetate); 3.58 (m, 2H-26); 4.11 (d, H-7 β); 4.41 (m, H-16); 5.01 (m, H-3).

(25R)-5-spirosten-3 β -ol-3-p-toluen-sulfonate (V)

To a solution of 5 g diosgenin (12 mmol) solved in 70 mL dry pyridine, 5.9 g (TsCl, 31.2 mmol) tosyl chloride are added. The reacting mixture is settled for 20 h at room temperature. After this period, it is poured on a KHCO_3 5 % aqueous solution with ice. The obtained tosylate is collected by suction and washed with water. The precipitate is recrystallized of a mixture of petroleum ether-ethyl acetate to obtain 6 g. T.f. 174 °C. FTIR (cm^{-1} , v, KBr) 1 588, 1 360, 1 170, 980, 920, 99, 860.

(25R)-3 α ,5-cyclo-spirostan-6 β -ol (VI)

A mixture constituted by 500 mg V (0.08 mmol), 490 mg AcOK, 10 mL acetone and 2 mL water is refluxed for 20 h. After this time, the reacting mixture is poured on iced water and the solid that precipitates is purified through column chromatography to obtain 230 mg VI. T.f. 166 °C. FTIR (cm^{-1} , v, KBr) 3 473, 1 444, 1 368, 1 072, 1 050, 970, 920, 896, 854.

(25R)-3 α ,5-cyclo-spirostan-6-one (VII)

Fifteen mL water and 1.47 g (17.6 mmol) NaHCO_3 were added to 5 mg (8.8 mmol) tosylate V solved in 90 mL butanone. Stirring for 5 h refluxes the reacting mixture; it is cooled at 5 °C and 2 mL Jones reagent (solution 8N CrO_3 in H_2SO_4) are added drop by drop. The stirring is performed for 3 h at the same temperature. After adding 15 mL NaCl 15 % aqueous solution, the organic phase is removed and washed with a NaHCO_3 (15 mL) and a NaCl (15 mL) aqueous solution, respectively. The organic phase is dried with MgSO_4 and vacuum-concentrated. The raw product is purified through column chromatography. It is obtained 2.99 g de VII. T.f. 178-179. FTIR (cm^{-1} , v, KBr) 1 674, 1 064, 1 043, 976, 924, 890, 856. MNR- ^1H (δ , ppm) 0.79 (d, CH_3 -27); 0.82 (s, CH_3 -18); 0.98 (d, CH_3 -21); 1.03 (s, CH_3 -19); 2.46 (dd, H-7 β); 3.47 (m, 2H-26); 4.4 (m, H-16).

(25R)-5 α ,2-spirosten-6-one (VIII)

The VII ketone (2.5 g; 6.06 mmol) is solved in 10 mL dimethylformamide (DMF) and treated with 40 mg *p*-toluensulfonic acid (*p*-TSA) and 50 mg NaBr at reflux with stirring for 3 h. The reacting mixture is cooled at room temperature and 25 mL ethyl acetate are added. The organic phase is washed with a NaHCO₃ (3x15 mL) aqueous solution and then with a NaCl (5 mL) and water solution. The organic phase is dried on Na₂SO₄ and vacuum-concentrated. The raw product is purified by through column chromatography to obtain 1.87 g de VIII. T.f. 203-204 °C. FTIR (cm⁻¹, v, KBr) 2 949, 1 708, 1 455, 1 381, 1 054, 996, 897. MNR-¹H (δ , ppm) 0.71 (s, CH₃-19); 0.77 (s, CH₃-18); 0.96 (d, CH₃-21); 2.3 (m, H-7 β ; H-5 α); 3.4 (m, 2H-26); 4.4 (m, H-16); 5.6 (m, H-2; H-3).

(25R)-5 α -spirostan-2 β ,3 β -dihydroxi-6-one-2-acetate (IX)

The VIII compound (1.5 g; 3.64 mmol) is solved in 120 mL AcOH and then water (3 mL) and 2 g (12 mmol) AgAcO. Subsequently, in a 15 min period, 1.55 g (6.13 mmol) I₂, finely powdered is added. The reacting mixture is stirred and heated at 50-60 °C for 3 h. It is settled and cooled at room temperature. It is filtered and the residue is washed with chloroform. The filtrate is diluted with 50 mL CHCl₃. Then, it is washed with water (2 x 50 mL) and with a NaHCO₃ (2 x 50 mL) aqueous solution. The organic phase is dried and the solvent is evaporated at reduced pressure. The raw product obtained is purified through column chromatography to obtain 1.5 g of IX. T.f. 209-210 °C. FTIR (cm⁻¹, v, KBr) 3 528, 2 948, 1 721, 1 250, 1 063, 978, 898. MNR-¹H (δ , ppm) 0.73 (s, CH₃-18); 0.76 (d, CH₃-27); 0.86 (s, CH₃-19); 0.94 (d, CH₃-21); 2.05 (s, CH₃-acetate); 2.3 (dd, H-5 α); 2.3 (d, H-7); 3.31 (t, H-3; H-26 α); 3.44 (m, H-26 β); 3.63 (m, H-3 α); 4.32 (m, H-16 α); 5.11 (m, H-2 α).

(25R)-5 α -spirostan-2 β ,3 β -dihydroxi-6-one-2,3-diacetate (X)

One g IX is solved in 200 mL pyridine and 10 mL Ac₂O. The procedure is similar to the one used for obtaining I. It is obtained 1 g of X. T.f. 198-199 °C. FTIR (cm⁻¹, v, KBr) 2 946, 1 741, 1 456, 1 369, 1 242, 1 054, 984, 918, 903. MNR-¹H (δ , ppm) 0.73 (s, CH₃-18); 0.76 (CH₃-27); 0.91 (s, CH₃-19); 0.95 (d, CH₃-21); 1.97 y 2.04 (s, CH₃-acetate); 2.33 (d, H-7); 3.35

(t, H-26 α); 3.45 (m, H-26 β); 4.42 (m, H-16 α); 4.75 (m, H-3 α); 5.25 (m, H-2 α).

(25R)-5 α -spirostan-7 α ,23 α -dibromo-2 β ,3 β -dihydroxi-6-one-2,3-diacetate (XI)

A solution of Br₂ (0,5 g; 0,4 mL; 0,94 mmol) in AcOH (1,5 mL) was added to a solution of X (0,5 g; 0,94 mmol) in 10 mL AcOH containing HBr traces. The reacting mixture is heated at 50 °C, with stirring for 2 h. It is cooled at room temperature and is poured on iced water. The resulting solid is filtered by suction and purified through column chromatography. It is obtained 0,4 g of XI. T.f. 150 °C. FTIR (cm⁻¹, v, KBr) 2 956, 1 743, 1 456, 1 369, 1 249, 1 053, 988, 951. MNR-¹H (δ , ppm) 0,79 (s, CH₃-18); 0,8 (d, CH₃-27); 0,9 (s, CH₃-19); 0,95 (d, CH₃-21); 2,05 y 2,1 (s, CH₃- acetates); 2,6 (t, h-20); 3,45 (m, 2H-26); 4,1 (dd, H-23 β); 4,25 (d, H-7 β); 4,5 (m, H-16); 4,95 (m, H-3 α); 5,3 (m, H-2 α).

MNR-¹³C spectroscopic data for some synthesized steroidal derivatives are reported (Table 1).

RESULTS AND DISCUSSION

Alternative ways of synthesis to obtain the different compounds are resumed in a general scheme (Fig. 1).

At present, many ecdysteroids and analogs have been synthesized from different substrates (phytosterols mixtures, bile acids, steroidal sapogenins, 6-keto.steroids, stygmasterol, steroidal 2,4-dien-6-ones and progesterone hormonal analogs and androstanes), but the yields and efficiency are not satisfactory what limits the commercial scaling of those derivatives with great perspectives for the comprehensive control of pests. In the last 10 years, the research of R+D+A have been oriented to the design, modeling and synthesis of ecdysteroidal analogs with structural variations in the C-17 lateral chain. To this effect, the steroidal sapogenins (diosgenin) can be a raw material of extraordinary importance and accessibility for obtaining advanced intermediates and ecdysteroidal analogs with a simplified lateral chain.

Moreover, diosgenin (25R-spirost-5-en-3 β -ol) has, in C-22, an oxygenated function and their natural analogs are biosynthetically related to polyhydroxylated steroids in the lateral chain, compounds with recognized hormone activity in the veg-

etal kingdom and among invertebrates.

Obtainment of the derived bromine IV

Diosgenin is treated with acetic anhydride (Ac₂O) in pyridine to obtain the I compound with a 95 % yield. When reacting I with meta-chloroperbenzoic acid, a mixture of a/b II epoxides is obtained with a total yield of 85 %. This mixture, without previous removal and solved in hot acetone, was subjected to an oxidative opening by treatment with a Chrome trioxide (CrO₃) aqueous solution to generate ketol III with a high yield (90 %). Those results agree with those reported by Swartz *et al.*,³⁴ higher than those described by Tacoronte *et al.*^{35,36} that employed bentonite without hydrothermal treatment as catalyzer and non-pollutant oxidant agent. When treating ketol III with Br₂ solved in acetic acid, according to the technique described³⁷ and modified, the IV derivative is obtained with a splendid yield (90 %).

Obtainment of the derived bromine XI

To obtain this derived dibromine diosgenin tosylate V synthesized by classic way (87.5 %) was used. The treatment of V with Potassium acetate in acetone with reflux originates the I-steroid VI with 65 %. This derivative is transformed in the VII ketone through oxidation with Jones reagent at low temperatures. In a search for oxidative alternatives for obtaining this derivative, diverse techniques were developed using different oxidant agents TEMPO type, heteropolyacids supported on silica gel, Collins reagents, pyridinium chlorochromate and others, but the yields reached were not higher than 50 %, so its synthetic interest was not relevant. At the same time, this derivative was obtained starting from diosgenin tosylate with a yield of 80 %.³⁸ When the ketone VII is solved in dimethylformamide and made react with NaBr in the presence of *p*-TSA with reflux, an isomerization process occurs that originates a mixture of two alkenes and the product VIII is the main derivative with a yield of 75 %. The diacetate X is obtained when the keto-olefin VIII reacts with silver acetate in aqueous AcOH in the presence of I₂ and heating (Prevost-Woodward conditions). This generates the intermediate IX (85 %) that is acetylated

Table 1. Chemical shifts ^{13}C (ppm, d, 25 °C) of the most significant synthesized derivatives.

Number of carbon	Compounds					
	II	III	IV	VIII	X	XI
1	32.1	29.5	30.6	39.1	40.8	40.8
2	27.1	26.5	26.0	124.4	68.6	68.4
3	71.3	70.8	69.9	124.9	71.6	71.2
4	36.0	32.3	32.8	21.7	21.5	24.4
5	65.1	80.1	81.2	53.8	56.8	50.1
6	58.9	212.2	204.0	211.4	209.2	202.7
7	28.9	41.8	52.3	47.0	46.3	57.7
8	29.4	36.8	38.0	37.1	36.8	46.6
9	42.4	44.3	37.4	53.3	54.4	40.4
10	35.1	42.5	41.9	39.9	40.6	42.5
11	20.4	21.2	20.5	20.9	21.3	21.3
12	39.3	39.5	38.7	39.5	39.3	38.8
13	40.2	41.1	41.8	40.7	40.9	42.5
14	56.5	56.1	52.1	55.4	56.1	51.9
15	31.6	31.6	52.5	31.5	31.4	30.6
16	80.6	80.5	80.2	80.3	80.3	80.5
17	61.8	62.0	63.3	62.0	61.9	60.9
18	16.2	16.8	18.1	16.3	16.3	16.2
19	15.9	14.0	13.9	13.5	16.0	14.5
20	41.5	41.6	39.7	41.5	41.3	41.3
21	14.4	14.4	15.4	14.4	14.0	14.0
22	109.2	109.3	109.2	109.2	109.2	108.8
23	31.3	31.4	0.6	31.3	31.2	49.5
24	28.8	28.8	28.8	28.7	28.6	40.5
25	30.2	30.3	30.4	30.2	30.1	30.0
26	66.8	66.4	6.5	66.6	66.8	66.7
27	17.1	17.1	17.3	17.0	17.0	17.0
CH ₃ -CO-O	21.3	21.4	21.3		20.9 - 21.1	20.9 - 21.1
	170.2	171.2	170.9		170.0 - 170.1	170.0 - 170.1

with Ac₂O. This diacetylated derivative global yield reaches 92.5 %.

The bromine derivative XI is obtained with a 62.5 % yield starting from X through Br₂ in acetic acid with HBr traces. The attempts of using ammonium polybrominated salts as smooth and selective agents in biphasic condition at room temperature were not satisfactory since the rupture of the spirocetallic molecular fragment and the hydroxyl group removal are observed.

Spectroscopic characterization

The spectroscopic data reported corroborate the proposed structures. The FTIR spectra show the characteristic bands in zone of 1 350-850 cm⁻¹ because of proper vibrations of the spirocetallic system associated to the narrowing of the C-C and C-O bonds what indicates that there

were not degradative structural changes in the E and F rings. All the synthesized compounds belong to the 25R series and this is corroborated because the 880-899 cm⁻¹ band is approximately twice more intense than the 925-910 cm⁻¹ band. The 1 713-1 735 cm⁻¹ frequency zone, corresponding to the acetoxi CH₃-CO-O- group is typical for the synthesized aceto-derivatives (**I**, **II**, **III**, **IV**, **IX**, **X**, **XI**). The 1 360 y 1 170-1 175 cm⁻¹ band corresponding to the asymmetrical and symmetrical vibrations of the SO₂ (ν_{SO₂S} y as.) group is characteristic for the tosyl derivative. The 1 588 cm⁻¹ band, assignable to aromatic ν_{C=C} corresponds to this aggrupation. The 3 473-3 500 cm⁻¹ band corresponding to the ν_{OH} of the hydroxyl group in C-6 is typical for the 3α-5 derivative cycle **VI**. For the **VII** compound, a steroidal ketone, the sign

corresponding to the n C=O vibration (6-oxo group) appears at n lower than 1 700 cm⁻¹ (1 674 cm⁻¹) because of conjugation interactions with the exocyclic cyclopropanic ring.

For the **VIII-XI** derivatives, the FTIR (cm⁻¹) signals corresponding to the different functional groups and their transformations are detailed in other papers.^{27,35,36} and they corroborate the proposed structures.

The MNR-¹H y ¹³C spectroscopy resulted very informative to facilitate the structural elucidation of the synthesized compounds. Among the common significant signals, the signal of the (CH₃) 18, 19, 21 and 27 methyl groups protons and the signal of the H₂-26 y H-16α protons. The H₂-26 signal is complex and corresponds to two chemical shifts (H-26α and H-26β) and practically does not change in the series of the studied compounds. H-26α (axial) is a

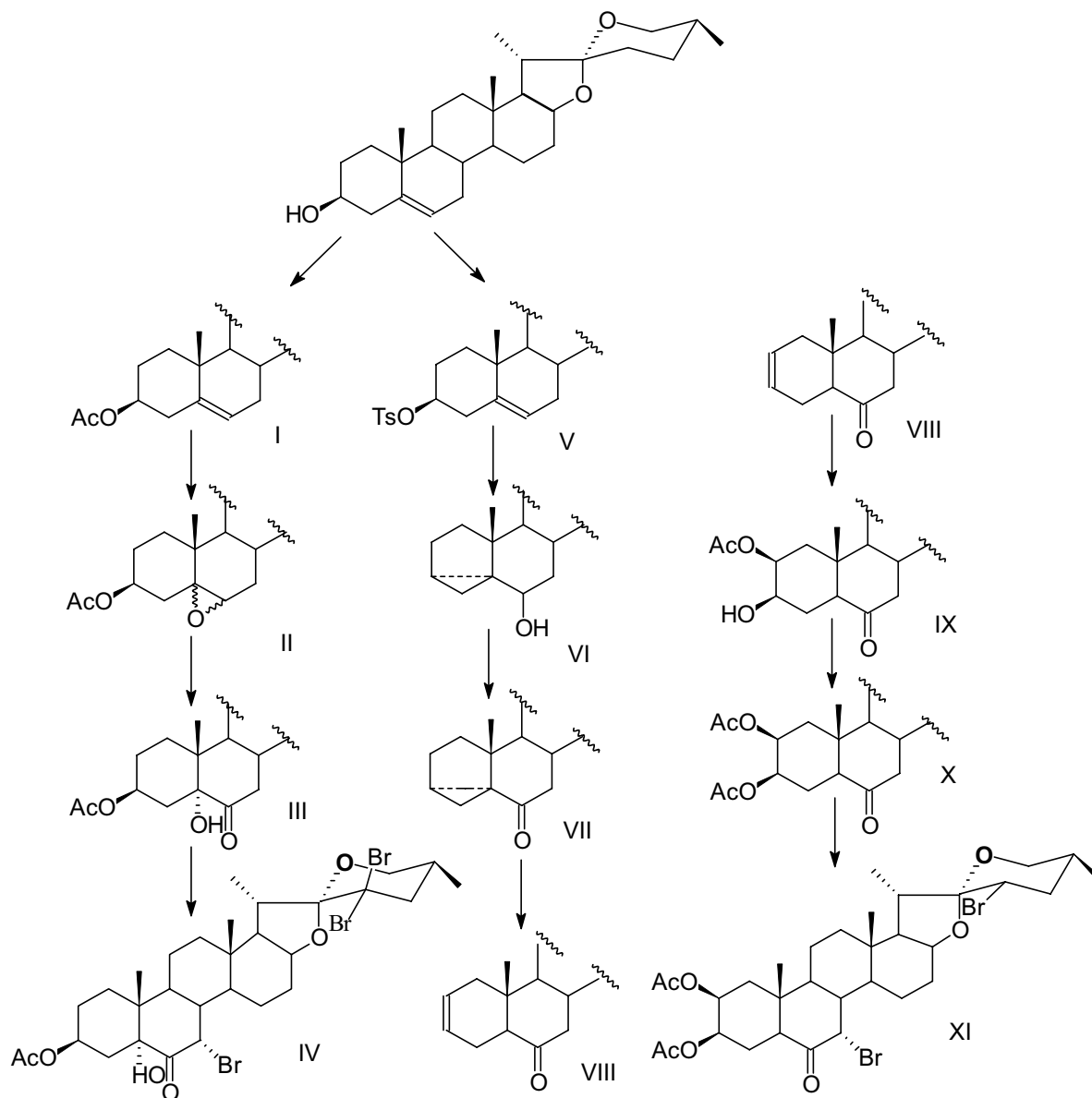


Fig. 1. Scheme of reactions developed for obtaining bromo-derivatives from diosgenin.

triplet with $\delta = 3.32$ ppm and its multiplicity is owed to a quasi-degenerated geminal and vicinal axial-axial double coupling ($J^2 \cong J^3 = 10.6$ Hz).

H-26 β is a double doublet with $\delta = 3.5$ ppm. Its multiplicity is owed to the geminal coupling ($J^2 = 10.6$ Hz) and equatorial-axial vicinal ($J^3_{ea} = 2.6$ Hz). For H-16 α , with a chemical shift of $\delta = 4.4$ ppm, the appearance of the double doublet is typical because of the coupling with the 15 β (equatorial), 15 α y 17 β (quasi-equatorial) 15 α y 17 β protons. In compounds presenting the 3 α ,5-ciclo-6-one system, the H₂-4 signals are more unblinded than in the compounds presenting the 3 α ,5-ciclo-6-ol system. The signal corresponding to the H-7 β (equatorial) appears as a double doublet ($J^2 = 15.9$ Hz, $J^3 = 4$ Hz) in 2.4 ppm. In MNR-¹³C, the signal for C-4 appears out of the common

range of chemical shift for the methylene groups (-CH₂-) of steroids, oscillating from 20-41 ppm.

In Δ^2 -6-oxo steroidal (VIII) compounds the signals of the H-2 and H-3 olefinic protons with $\delta = 5.5$ y 5.7 ppm, respectively. The 7 β y 5 α protons signals appear superposed in 2.3-2.4 ppm. In MNR-¹³C the C-2 and C-3 signals are unblinded to $\delta = 124.4$ and 124.5 ppm, respectively, while C-4 is blinded to 21.7 ppm. The most significant signals for the intermediate IX in MNR-¹H appear at $\delta = 5.1$ y 3.6 ppm corresponding to the chemical shifts of the H-2 α and H-3 α protons (both multiplets). In MNR-¹³C, the signals corresponding to C-2 and C-3 are blinded to 71.9 and 70.4, respectively. When acetylating this hydroxyl, compound X, an unblinding of H-3 α is observed to 4.75 ppm. A similar effect is ob-

served for C-3 because of the existence of a shift from 70.4 to 71.6 ppm. For the compound XI, a signal is observed about 4.2 ppm (doublet) belonging to the equatorial H-7 β . The existence of a signal (double doublet) about 4.05 ppm, corresponding to the axial H-23 β , indicates the presence of an equatorial Br atom in that position. For IV, this signal is not observed, what indicates, *a priori*, the disubstitution in C-23. The MNR-¹³C results corroborate what was mentioned before when unblindings were observed in C-7 and C-23.

Regarding the compound II, the most important signals in the MNR-¹H spectrum correspond to the H-6 α y H-6 β protons, doublets, appearing at $\delta = 3.07$ and 2.87 ppm. Those signals show that there is a mixture of alpha and beta epoxides. This in-

intermediate oxidative opening originates the ketone **III**. The significant change can be observed in the MNR-¹³C spectrum for C-5 and C-6 in **III**. Those signals are unblinded from 65.1 to 80.0 ppm and 58.9 to 212.2 ppm, respectively.

Preliminary studies on the synthesized compound biological activity

Because of the extraordinary structural similitude between the spirostane starting substrates and their derivatives with the natural ecdysteroids, it was evident the necessity of valuing the potential biological properties for this series of analogs. *A priori* it was considered that the bromo-derivatives could be an excellent and promissory source of potentially active agents as insecticides. In the laboratories for pest control of the Institute of Tropical Medicine "Pedro Kouri", several tests were developed for determining the insecticide action of the bromine derivatives **IV** and **XI** obtained from diosgenin. It was demonstrated that those intermediates have a notorious biological activity on *Blattella germanica* or German cockroach, a pest of wide distribution and carrier of diseases. The compounds, tested at different concentrations and employing different techniques (topical use and food addition) retarded the emergence of adults and morphogenetic effects on the existence of a functional Br group were observed in the spirocetallic ring that block the interactions with the specific receptor-ecdysteroid-white metabolic process. At present, tests for determining the residual genetic effect are developed.

CONCLUSIONS

The possibility of using diosgenin as raw material to obtain advanced bromine intermediates of ecdysteroids is confirmed. The proposed synthetic design allowed, at laboratory stage, to elaborate a sequence of reactions that uses

standardized reagents and reaction parameters and demand a minimal energy consumption as well as the non-use of extreme control and safety measures, what makes the process ecologically acceptable. The bromine derivatives were characterized through MNR and FTIR techniques and the proposed structures were corroborated. The synthesized substituted bromo-intermediates showed a pesticide activity against the *Blattella germanica* species, a pest widely expanded in the human communities.

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