Tetrahedron. Vol. 30, pp. 3587 to 3589. Pergamon Press 1974. Printed in Great Britain

THE STRUCTURE OF SYRIOGENIN

C. CASAGRANDE,[a] F. RONCHETTI[b] and G. RUSSO*[b]

[a] Simes S.p.A.

[b] Institute of Organic Chemistry of University of Milan, Via Saldini 50, 20133 Milano, Italy

(*Received in the UK* 3 *April* 1974; *Accepted for publication* 2 *May* 1974)

Abstract—Syriogenin was demonstrated by chemical and spectroscopic evidence to have the structure 12β-hydroxyuzarigenin and confirmed by transformation into 3,12-diketo-5α,14α-etianic acid methyl ester.

In 1962 L. Masler *et al*[1] assigned to the genin syriogenin, isolated from *Asclepias syriaca L.*, the structure (1) of 3β,12β,14β-trihydroxy-5α-$\Delta^{20(22)}$-cardenolide on the basis of IR and UV spectra, of reactivity toward acetylation and of molar rotation differences.

However, Reichstein *et al*[2] isolated the same compound from *Calotropis procera R. Br.*, and, on the basis of the mass-spectral data, excluded the location of an OH group at C-12, proposed by Masler *et al.*

From the roots of *Dorstenia contrajerva* (Moraceae) of Mexico we isolated a compound $C_{23}H_{34}O_5$ which was shown to be identical (IR, m.p., $[\alpha]_D^{20}$) with syriogenin of Masler, and whose mass spectrum corresponded to that published by Reichstein.[2] We decided therefore to demonstrate the structure of syriogenin by unequivocal chemical proof.

Chemical evidence published by the above authors show that two OH groups of syriogenin are secondary (Ac_2O-pyridine easily affords a diacetate (2)[1]) and that no 1,2-glycol group is present (negative $NaJO_4$/benzidine test[2]); so, one secondary OH group should be located at C-3 on biogenetic grounds, and the other in one of the following positions: 1, 6, 7, 11, 12, 16. Jones' oxidation of syriogenin affords a diketone (3) whose UV spectrum (λ_{max} 225 mμ, $\log \epsilon = 4{\cdot}00$; 270 mμ, $\log \epsilon = 3{\cdot}44$) remains unchanged by addition of alkali; the lack of bathochromic shift, ruling out the presence of an α- or β-dicarbonyl system, confirmed the exclusion of positions 1,2 or 4 for the second ketonic function.

Dehydration of the diketone (3) under drastic conditions (HCl/AcOH at reflux)[3] yields the unsaturated derivative (4), the UV spectrum of which lacks in the typical absorption of α,β-unsaturated ketones, indicating that the second ketonic group cannot be located in 7 or 16 positions.

The NMR spectrum of diacetate 2 shows at 4·60 δ the typical signal of the 3α-hydrogen in 3β-acetoxy-5α-steroids, surmounted by a couple of doublets (X part of an ABX system), which can be attributed to the 12α-axial hydrogen of a 12β acetoxysteroid. This system appears also in the NMR spectrum of digoxigenin diacetate (5), whereas the axial hydrogen of a 6α-acetoxy or a 11α-acetoxy-compound is expected to give rise to a more complex signal,[4] being flanked by three H atoms.

The above data suggest the structure of 12β-hydroxy-uzarigenin proposed by Masler *et al.*[1] as correct.

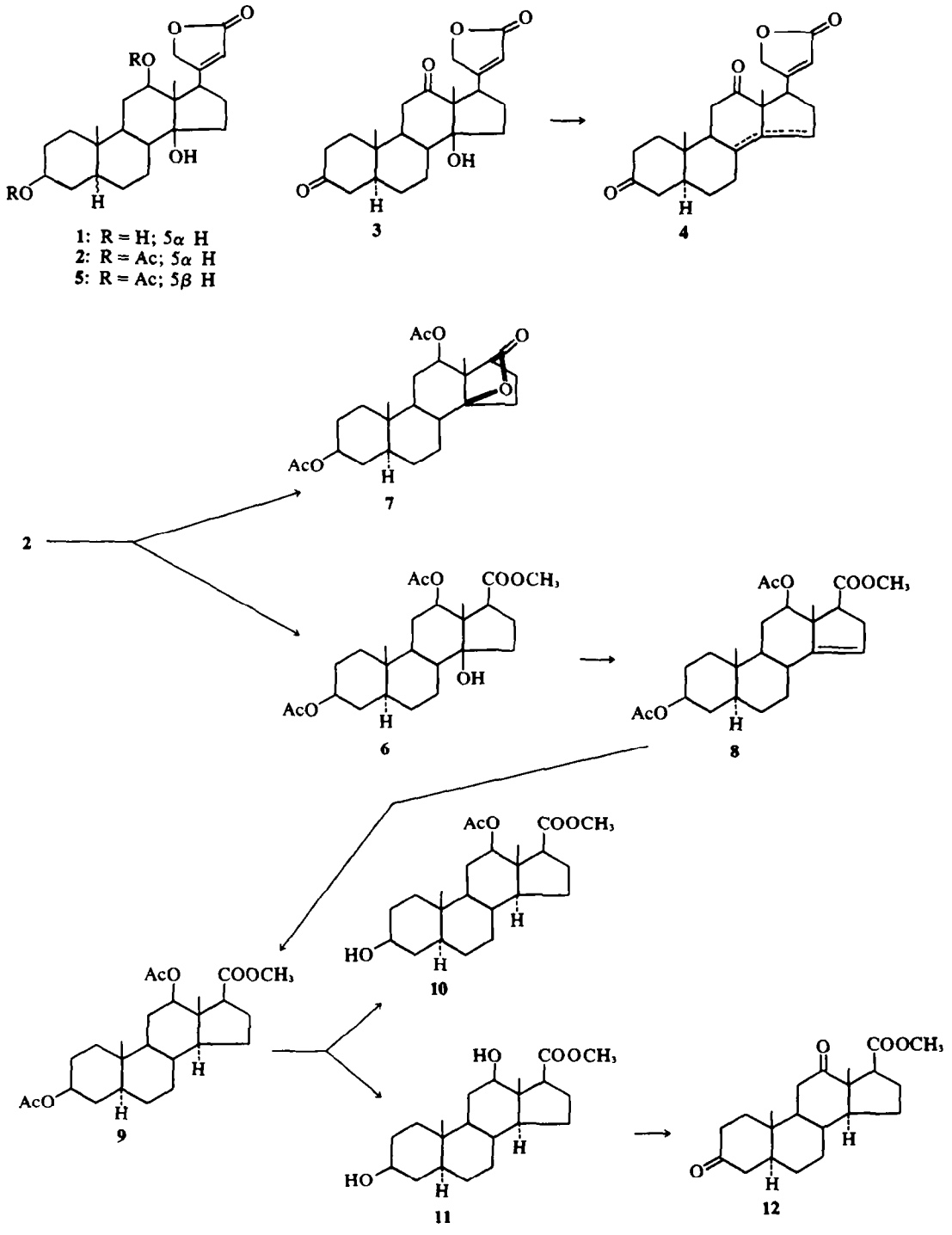
A proof of the location at C-12 of the second OH group was finally obtained by degradation of syriogenin to the corresponding diketoetianic acid methylester 12 (Scheme 1), which was found identical with an authentic sample of 3,12-diketo-5α,14α-etianic acid methylester.[5]

EXPERIMENTAL

Isolation of syriogenin (1). Powdered dried roots (800 g) of *Dorstenia contrajerva* were extracted 5 times at 40° with 9 l. of $CHCl_3$–EtOH (1:1). The extracts were evaporated and the residue was taken up in MeOH (500 ml); acetone (2·5 l) was added and soln was filtered and evaporated. The residue was redissolved in 70% aqueous EtOH (900 ml); a soln of basic lead acetate was added in portions until no ppt was formed on further addition; the soln was filtered, excess lead was removed by addition of ammonium phosphate soln and further filtration; the filtrate was extracted with $CHCl_3$ (3 × 800 ml). The extracts were evaporated, the residue was triturated in ether and dried, yielding 32 g of a cream white powder. This (30 g) was chromatographed on a silica gel (Merck, 0·05–0·2; 1·7 Kg) column eluting with EtOAc (10 l.) and then with EtOAc–EtOH (85:15) in one-liter fractions. Fractions 2–6 upon evaporation to 1 l. yielded a crystalline ppt (5·20 g), which was recrystallized from EtOAc, giving 2·35 g of syriogenin, m.p. 268–270°: $[\alpha]_D^{20} = +9{\cdot}0$ ($c = 1$, py).

The IR spectrum is superimposable with that published by Masler *et al.*[1] The mass-spectrum exhibits the same fragmentation pattern of syriogenin isolated by Reichstein.[2]

Oxidation of syriogenin. Syriogenin (100 mg) dissolved in 15 ml acetone, were treated with 0·07 ml Jones' reagent at r.t. After 2 h a few drops of EtOH were added, and the mixture was poured into H_2O and extracted with $CHCl_3$:MeOH(9:1, v/v); the organic phase, washed with H_2O, dried on Na_2SO_4 and evaporated *in vacuo*, afforded



87 mg of crude 3, which was crystallized from $CHCl_3$–MeOH, m.p. 251–2°; $[\alpha]_D^{20} = +81°$ ($c = 1$, py); ν_{max} (Nujol): 3520, 1805, 1740, 1705, 1625 cm^{-1}; λ_{max}^{MeOH}: 224 nm ($\log \epsilon = 3{\cdot}96$) *and* 270 *nm* ($\log \epsilon = 2{\cdot}80$). The UV spectrum remained unchanged when ion in 0·1N NaOH/MeOH. MS: 386 (M^+), 372, 368, 340, 325, 301, 283, 263, 230, 219, 215, 207, 176, 164, 163, 135, 121, 111 *m/e*.

Dehydration of the diketone (3) *to* (4). Diketone 3 (26 mg) was dissolved in a mixture of 5·5 ml AcOH and 0·3 ml conc HCl. After 30′ at reflux the mixture was

evaporated *in vacuo* and the crude product purified by chromatography over 2·5 g of Al_2O_3 (eluent $CHCl_3$). The UV spectrum of the combined $CHCl_3$ eluates presented the band of the butenolide group at 225 nm, but lacked in the typical absorption of α,β-unsaturated ketones.

Diacetylsyriogenin (**2**). Syriogenin (100 mg) was suspended in 20 ml pyridine and treated with 2 ml Ac_2O; after stirring at r.t. for 2 h the solid dissolved; after 15 h the soln was poured into water and extracted with $CHCl_3$; the organic phase was washed with dil HCl, H_2O, dil NaOH, H_2O, dried and evaporated to yield 105 mg of crude **2**, which was crystallized from benzene-isopropyl ether; m.p. 170–2°; ν_{max}(Nujol) = 3500, 1805, 1740, 1620 cm^{-1}; $[\alpha]_D^{20}$ = +32°, c = 1 in $CHCl_3$). NMR ($CDCl_3$): 0·83 (s, 3H, —CH_3); 0·90 (s, 3H, —CH_3); 2·01 (s, 3H, —OCOCH_3); 2·08 (s, 3H, —OCOCH_3); 4·62 (dd; δ_1 = 4·55, J_1 = 5Hz, δ_2 = 4·68, J_2 = 5 Hz; 1 H, —CHOAc); 4·60 (m; 1 H; —CHOAc); 4·82 (t, J = 2 Hz; 2 H, CH_2OCO—); 5·84 (t, J = 2 Hz; 1 H, —CH=).

Degradation of diacetylsyriogenin (**3**) *with* $KMnO_4$. Diacetylsyriogenin (935 mg), dissolved in 60 ml acetone, was treated with 935 mg $KMnO_4$ for 2 h at r.t.

The mixture was evaporated *in vacuo* at r.t., 10 ml of HCl 0·5 N was added and the organic phase was extracted repeatedly with Et_2O; the Et_2O extracts were extracted with 0·2N NaOH. The residual ethereal phase, washed, dried and evaporated, afforded 470 mg of neutral products which were re-oxidized with $KMnO_4$. From the alkaline extracts, acidified with dil HCl, Et_2O extracted 146 mg of acidic products. Two re-cycles of the neutral phase afforded, respectively, 86 and 16 mg of acidic products.

The combined acidic products (248 mg) were dissolved in 20 ml MeOH and treated with excess CH_2N_2 in ethereal soln. Evaporation of the solvent, acetylation with Ac_2O/Py, and recovering of products with usual work-up afforded 311 mg of crude product which was chromatographed on 30 g of SiO_2-celite (1/1, v/v); benzene-EtOAc (98:2, v/v) eluted 147 mg of pure **6**, oily product, ν_{max}($CHCl_3$): 3450, 1725, 1370 cm^{-1}; benzene-EtOAc (9:1, v/v) eluted 145 mg of an impure compound whose IR spectrum showed bands at 1780 and 1730 cm^{-1}, typical of a lactone-ester (probably **7**).

Dehydration of (**6**). Compound **6** (147 mg) dissolved in 3 ml pyridine, was treated at 0° with 0·25 ml $SOCl_2$ in 1·25 ml pyridine for 16 h The mixture was poured into H_2O and extracted with $CHCl_3$; usual work-up of the chloroform extracts afforded 138 mg of crude product. Chromatography on SiO_2-celite (1/1, v/v) with benzene-EtOAc (99:1, v/v) as eluent yielded 121 mg of **8**, oily product, ν_{max} ($CHCl_3$): 1730, 1610, 1380 cm^{-1}; NMR ($CDCl_3$): 0·81 (s, 3H, —CH_3); 0·97 (s, 3H, —CH_3); 1·98 (s, 3H, —OCOCH_3); 2·02 (s, 3H, —OCOCH_3); 4·80 (m, 1H, —CHOAc); 4·82 (dd, δ_1 = 4·76; J_1 = 5Hz; δ_2 = 4·95; J_2 = 5Hz; 1H, —CHOAc); 5·20 (t, 1H, —C≡C—H).

Hydrogenation of (**8**). Compound **8** (121 mg) in 3 ml AcOH–H_2O (9:1) was reduced with H_2 at atm press over 45 mg of pre-reduced PtO_2. After 1 h the theoretical amount of H_2 was absorbed and the soln was filtered and evaporated *in vacuo*. The crude product was chromatographed on 12 g of SiO_2-celite: benzene-EtOAc (9:1, v/v) eluted 107 mg of **9**, oily product, ν_{max} ($CHCl_3$): 1730, 1380 cm^{-1}; NMR ($CDCl_3$): 0·80 (s, 3H, —CH_3); 0·96 (s, 3H, —CH_3); 1·96 (s, 3H, —OCOCH_3); 2·00 (s, 3H, —OCOCH_3); 3·64 (s, 3H, —COOCH_3); 4·80 (dd; δ_1 = 4·77; J_1 = 5Hz; δ_2 = 4·85; J_2 = 5Hz; 1H, —CHOAc); 4·80 (m, 1H, —CHOAc).

Hydrolysis of **9**. Compound **9** (104 mg) dissolved in 3 ml MeOH, was treated with 3 ml 2N K_2CO_3 under N_2, and stirred at r.t. After 24 h, the mixture was poured into water, acidified with dil HCl and extracted with $CHCl_3$. The residue of the organic phase was dissolved in MeOH and treated with ethereal CH_2N_2. Evaporation to dryness left 98 mg crude product, which was chromatographed on 10 g of SiO_2-celite (1:1, v/v): using benzene-EtOAc (8:, v/v) as eluant, yielding **10** (82 mg) as the less polar compound and **11** (12 mg) as the more polar diol. The structure of methyl 3β-acetoxy-12β-hydroxyandrostan-17β-oate was assigned to **10** on the basis of NMR spectrum ($CDCl_3$): 0·82 (s, 3H, —CH_3); 0·85 (s, 3H, —CH_3); 1·98 (s, 3H, —OCOCH_3); 3·6 (s, 3H, —COOCH_3); 3·68 (m, 1H, —CHOH); 4·78 (dd, δ_1 = 4·72, J_1 = 5Hz; δ_2 = 4·83, J_2 = 5Hz; 1H, CHOAc).

The diol (**11**) was crystallized from MeOH, m.p. 145–6°; $[\alpha]_D^{20}$ = +38·8° (c = 1, Py); ν_{max} (nuyol): 3400, 1740 cm^{-1}.

The monoacetate (**10**) was again submitted to hydrolysis as above; the reaction was monitored by TLC (eluent benzene–EtOAc, 9:1), and was complete after 72 h at r.t. and 24 h at 40°.

Work-up as above afforded 78 mg of diol (**11**).

Oxidation on diol (**11**). Compound **11** (52 mg) was dissolved in 1 ml AcOH and treated with a soln of 50 mg of CrO_3 in 2·5 ml of AcOH. After 4 h at r.t. a few drops of EtOH were added, the mixture was poured in water and extracted with $CHCl_3$; the organic phase, washed with dil NaOH and H_2O, dried and evaporated *in vacuo*, left 38 mg of crude **12** which was crystallyzed from acetone-Et_2O, m.p. 203–5°, undepressed when mixed with an authentic specimen of 3,12-diketo-5α,14α-etianic acid methylester;[5] $[\alpha]_D^{20}$ = +138° (c = 0·8, $CHCl_3$). The IR spectrum was superimposable with that of the above sample.

Acknowledgements—We thank Prof. L. Canonica for its interest in this work and Prof. G. Severini Ricca for the N.M.R. spectra.

REFERENCES

[1]L. Masler, S. Bauer, O. Bauerova and D. Sikel, *Coll. Czechosl. Chem. Comm.* **27**, 872–895 (1962)
[2]F. Brüschweiler, W. Stöcklin, K. Stöckel and T. Reichstein, *Helv. Chem. Acta.* **52**, 2086 (1969)
[3]G. Juhasz, Ch. Tamm, *Ibid.* **44**, 1063 (1961)
[4]K. Tori, E. Kondo, *Steroids* **4**, 713 (1964)
[5]Kindly furnished by Prof. T. Reichstein