ON THE STRUCTURE OF RAMANONE Hiroshi Mitsuhashi and Taro Nomura Faculty of Pharmaceutical Sciences, Hokkaido University Sapporo, Japan

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<u>Metaplexis japonica</u> Makino (Japanese name, "Rama", Asclepiadacea family) is a plant widely distributed in Japan. Concerning the components of this plant in stems and roots, we had reported the isolation of sarcostin (1),^{1,2} metaplexigenin (II),³ benzoylramanone (III), m.p. 222-226°, $C_{28}H_{36}O_5$, and four other aglycones. Alkaline hydrolysis of III gave benzoic acid and a neutral fraction. The neutral fraction is a 4:1 equilibrium mixture of ramanone (IIIa), m.p. 184-194°, $C_{21}H_{32}O_4$, and isoramanone (IIIb), m.p. 220-234°, $C_{21}H_{32}O_4$.

Tschesche, <u>et al</u> isolated digipurpurogenin-II (IV) as a genin from the leaves of <u>Digitalis purpurea</u> and obtained isodigipurpurogenin-II^{6,7} by the alkali treatment of IV. We found IIIa to be identical with isodigipurpurogenin-II, and IIIb to be the same as digipurpurogenin-II by mixed m.p. determination. Tschesche proposed the structure IV for digipurpurogenin-II and the C-17 epimer for isodigipurpurogenin-II.⁸

The O.R.D. curve of IIIa showed a negative Cotton effect, trough $[a]_{301}$ -1306°, peak $[a]_{260}$ +1238° in MeOH, and IIIb showed a positive Cotten effect $[a]_{305}$ +895°, trough $[a]_{265}$ -659° in MeOH. The O.R.D. curve of III, peak $[a]_{305}$ +532°, trough $[a]_{270}$ -680° in MeOH, is very similar to that of IIIb, so it appears that the two compounds have the same configuration at C-17. IIIa was refluxed for 5 hr. with 9% MeOH-KOH and the resulting mixture was examined by paper chromatography. Its result indicated that

HO

(IV)

Ċ=0

ÓH





(Va) H at C-17=B (Vb) H at C-17= α

Ac0

HO

OH

HO

ÓH

ÇHz





Ĥ

IIIa is the main product and IIIb, a minor product. The same experiment with IIIb gave identical results. These observations suggest that IIIa and IIIb are epimeric at C-17. If we assume that IIIa and IIIb have a C/D trans juncture, the form stable to alkaline hydrolysis is a 17α -H type⁹ and this type of compound should show a strong positive Cotton effect.¹⁰

HC

Since ramanone (IIIa) is the main product upon alkaline hydrolysis, it must be more stable than IIIb. This assumption is inconsistent with O.R.D. results. On the other hand, for C/D <u>cis</u> steroids the more stable form is the 17B-H type and they show a negative Cotton effect.^{1,2} While the 17a-H type show a positive Cotton effect. The molecular amplitude of the 17B-H type is very similar to that of IIIa, and 17a-H type is similar to IIIb. Deacylcynanchogenin is easily isomerized in hot alkaline solution to give a 7:3 equilibrium mixture of Va and 17-isodeacylcynanchogenin.¹¹ The structures of compounds Va and Vb have been conclusively proven. The O.R.D. curve of Va showed a negative Cotton effect, with an amplitude of $a = -7400^{\circ}$, and its diacetate showed a remarkable shift of amplitude to the negative side, $a = -11000^{\circ}$. The same relation has been found, ramanone, $a = -8800^{\circ}$, and diacetylramanone, $a = -12000^{\circ}$.

Synthetic proof of these structures was attempted by the methods similar to that described by Plattner, <u>et al</u>.¹² Compound VII, prepared from hecogenin acetate (VI),¹³ was converted to the dienone (VIII), m.p. $152-154^{\circ}$, $C_{25}H_{34}O_5$, $[\alpha]_D^{17}+279^{\circ}$ (c=0.92, CHCl₃), UV_{maximum} at 304 mµ (loge= 4.16), by N-bromosuccinimide. Treatment of VIII with monoperphthalic acid afforded an epoxide (IX), m.p. 148-153°, $C_{25}H_{34}O_6$, $[\alpha]_D^{17}+88°$ (c= 0.92, CHCl₃), UV_{maximum} at 244 mµ (loge= 3.65). For the configuration of the epoxide ring, it seemed reasonable to assume that it had a ßepoxide ring, since Flattner, <u>et al</u>, reported the conversion of 5α-pregna-14,16-dien-3B-ol-20-one-3-acetate to its 14,15β-epoxide by the same reagent. The 12β-acetoxy group (IX) is equatorial, so it was assumed that this group does not affect the formation of epoxide ring. Moreover, the following facts are in agreement with this assumption. When IX was hydrogenated over $\frac{76}{2}$ Pd-BaSO₄ in EtOH, three crystalline substances, X, XI, and XII,

were obtained. X had m.p. 169-172°; C25H3806; VMujol 3650, 3500, 1750, 1710, 1690, 1270, 1235; no characteristic absorption was shown in UV spectrum; R.D. (in MeOH) trough [a]₃₀₄-1800°, peak [a]₂₅₅+1150°, a= -12500°. These data suggest that X is 5a,17a-pregnan-3B,12B,14B-triol-20-one-3,12-diacetate. On the other hand, diacetylramanone (IIIc) was hydrogenated using PtO2, then oxidized with CrO3 in acetic acid to dihydrodiacetylramanone, m.p. 169-171°, which was proved to be identical with X by mixed melting point and comparison of I.R. spectra. XI had m.p. 175-178°; C₂₅H₃₈O₅; R.D. (in MeOH) trough [a]₃₀₄-1185°, peak [a]₂₅₆+1297°, a= -9977°, and was also obtained from dienone VIII by hydrogenation with Pd-CaCO3. Hence its structure is 5a,14B,17a-pregnan-3B,12B-diol-20-one-3,12-diacetate.¹² XII showed m.p. 180-183⁶; C₂₅H₃₈O₆; neither a hydroxyl group nor a conjugated double bond was detected in an I.R. spectrum; R.D. (in MeOH) trough $[\alpha]_{302}$ -776°, peak $[\alpha]_{258}$ +942°, a= -7500°, and its N.M.R. spectrum had broad singlet at 6.75τ . (1 proton), characteristic for H on an epoxide ring (in CDCl₃, T.M.S. used as internal standard). On this basis, the structure of XII was assigned as being 5a,14B,17a-pregnan-14B,15B-epoxy-3B,12B-diol-20-one-3, 12-diacetate. For the comparison of O.R.D. data, 5a, 14B, 17a-pregnan-3B, 14B-diol-20-one-3-acetate (XIII), 5a,14B,17a-pregnan-3B-ol-20-one-3-

TABLE I

	O.R.D. DATA OF XIII,	XIV, AND XV	
Compound	Trough [a] ₃₀₂	Peak $[\alpha]_{259}$	a
XIII	-1140°	+1120 °	-8497 °
XIV	-715°	+1360 °	-7470 °
xv	-461.5°	+1038 °	-5617°

(In MeOH; a= molecular amplitude, amplitude x M.W./100)

acetate (XIV), and 5a,14B,17a-pregnan-14B,15B-epoxy-3B-ol-20-one-3-acetate

(XV) were synthesized by Mr. M. Fukuoka.

These results were consistent with those of X, XI, and XII. In view of the above facts, the most reasonable conclusion is that the structure of ramanone, isoramanone, and benzoylramanone correspond to IIIa, IIIb, and III, respectively.

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FOOTNOTE

Prof. R. Tschesche communicated to us his results by letter. (December 10, 1963).

The I.R. spectra of ramanone (isodigipurpurogenin-II) and isoramanone (digipurpurogenin-II) indicates that the hydroxy group in ramanone diacetate is non-hydrogenbonded in dilute solution, while isoramanone diacetate showed a strong intermolecular hydrogen bond. He had difficulty for the explanation of these phenomena by his formula, but these facts can be explained by our structure.