Isolation and Characterization of Fukujusonorone, a 18-Norpregnane Derivative from *Adonis amurensis* Regel et Radd[1]

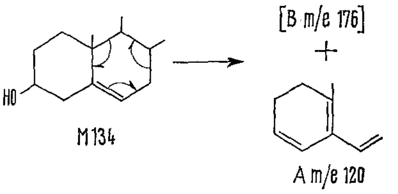
Adonis spp. (pheasants-eyes) have been used as folk medicines and are known to contain several cardiac glycocides[1]. In our earlier papers[2], we reported the isolation and characterization of various pregnane homologues from Japanese pheasants-eyes, *Adonis amurensis* Regel et Radd. This communication describes the isolation of a new aglycone, fukujusonorone, and the data which permit one to assign an 18-norpregnane structure I to it.

Fukujusonorone (I) was obtained by the column chromatography of the crude aglycone fraction and crystallized from acetone-water in hydrated crystals, mp 88–90 °C. Elemental analysis of fukujusone fitted an empirical formula, $C_{20}H_{26}O_3 \cdot 1/2\ H_2O$, which was also confirmed by the appearance of the molecular peak at m/e 314 in the mass spectrum. The IR-spectrum of I exhibits absorptions at 3600 (hydroxyl group), 1703 (isolated ketone), 1660 and 1615 cm^{-1} (α,β-unsaturated ketone). The presence of an α,β-unsaturated ketone was also supported by the UV-absorption at 250 nm ($\varepsilon =$ 9000). The NMR-spectrum shows signals for an angular methyl group (τ 8.90, 3H, singlet), a methyl ketone (τ 7.70, 3H, singlet), a methine protone (6.60, 1H, roughly resolved triplet), hydrogen adjacent to secondary hydroxyl group (τ 6.10), and an olefinic proton on a trisubstituted double bond (τ 4.60). The general feature of the spectrum strongly indicates that I is a derivative of pregnan-20-one which lacks one angular methyl group, and the pattern of the signals at τ 6.10 and 4.60 suggests the presence of 5-en-3β-ol system.

Acetylation of II gave a hydroxyl-free monoacetate $C_{22}H_{28}O_4$, mp 110–115°C, whose spectroscopic properties resemble those of I. Thus out of 3 oxygen functions, one is a secondary hydroxl group and 2 others are ketones. Moreover, since the chemical shift of the olefinic proton shows that the trisubstituted double bond does not participate in conjugation, there should be a tetrasubstituted double bond in conjugation with one of the ketones. The mass spectrum was most informative. The prominent peaks seen at m/e 105 and 120 are characteristic of 5-en-3β-ol steroids, formed through a retro-Diels-Alder process and dehydration. This indicated that the missing methyl group is not the 19-methyl group but 18-methyl group.

The fragments as m/e 271, 253 are evidently produced by the removal of an acetylinium ion, confirming the presence of a methyl ketone side chain at C-17. There are also prominent fragments, m/e 271 (M-42), m/e (M-42-43-18) formed by the loss of ketone, a fragmentation typical of an α,β-unsaturated ring ketone with an α-methylene structure. The occurrence of a fragment, m/e 211 (M-42-43-18) clearly distinguished the unsaturated ketone from the methyl ketone. It should also be noted that the same cleavage was observed with the retro-Diels-Alder fragment B, as peaks, m/e (B-42), m/e (B-43), m/e (B-42-43), illustrating again that the ketone functions are attached to ring C or D (scheme).

Since it is evident from the Ir-absorption (1660 cm^{-1}) that the unsaturated ketone is not a 5-membered one, the partial structure of Δ 13(14)-12-one was assumed. The compounds (i) and (ii) with analogous chromophores, reveal the UV- and IR-spectra similar to that of I[3]. An alternative structure of Δ 8(9)-11-one is less plausible, because the abnormality of the UV-absorption or instability in basic media expected from the peculiar Δ 5, 8(9)-dien-11-one system[4] was not observed. In this way, the structure of 3β-hydroxy-Δ 4, 13(14)-18-nor-



Scheme

m/e 272 (M-42), m/e 271 (M-43), m/e 254 (M-42-18), m/e 253 (M-43-18), m/e 211 (M-42-43-18), m/e 134 (B-42), m/e 133 (B-43), m/e 91 (M-42-43)

Mass fragmentation of fukujusonorone (I).

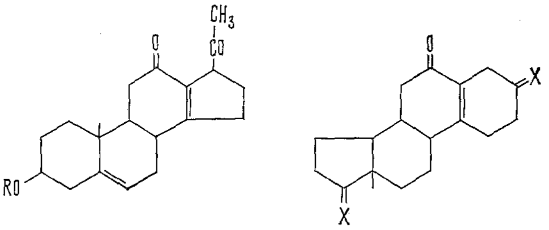
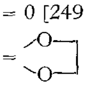
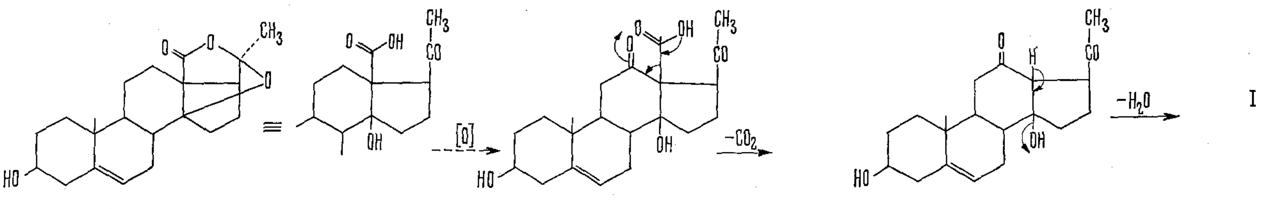


Chart 1

I. R = H [250 nm (9000) 1660, 1615 cm^{-1}]
II. R = Ac
III. R = Ac (17_yiso)
(i) X = 0 [249 nm (9650) 1673, 1623 cm^{-1}]
(ii) X =  [249 nm (11,900) 1685, 1619 cm^{-1}]



Adonilide

Chart. 2

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[1] Abstracted from the Symposium Papers of the 12th Symposium on the Chemistry of Natural Products, Sendai 1968, p. 174.

[2] Y. Shimizu, Y. Sato and H. Mitsuhashi, Chem. pharm. Bull., Tokyo *15*, 2005 (1967); Chem. pharm. Bull., Tokyo, submitted for publication.

[3] M. Amorosa, L. Caglioti, C. Cainelli, H. Immer, J. Keller, H. Wehrli, M. M. Lj. Mihailovic, K. Schaffner, D. Arigoni and O. Jeger, Helv. chim. Acta *45*, 2674 (1962).

[4] T. Masamune, M. Takasugi, H. Suzuki, S. Kawahara, M. Gohda and T. Irie, Bull. chem. Soc. Japan *35*, 1749 (1962).

pregnadiene-12,20-dienone was postulated as one which fulfils all the requirements. The proposed structure can also explain the unusually deshielded chemical shift of the methine protone at C-17. Treatment of I with dilute alkali gave an intensely yellow colored solution, from which, after acetylation, II and an isomeric acetate, III, were isolated. III has the same UV-absorption and mass spectrum as II and was considered to be the isomer at C-17 or less possibly at C-9[5].

Although the unequivocal elucidation of the structure has to wait for correlational experiments which are now under way, the authors believe that fukujusonorone is the first example of a genuine 18-norsteroid in nature[6]. While many 19-norsteroids are known, an 18-norsteroid has not been found. As for the biogenesis of I, the authors present the following sequence which involves β-ketoacid decarboxylation (or similar reaction) followed by β-elimination. Co-existence of adonilide (IV) and some 12,14-oxygenated pregnanes in the same plant may support this hypothesis.

Zusammenfassung. Aus den Wurzeln des japanischen Adonisröschens (*Adonis amurensis* Regel et Radd) wurde Fukujusonoron, ein neues Aglykon, das sich von 18-nor-Pregnan ableitet, isoliert.

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[5] Failure of transformation to the conjugated ene-dione system shows the deconprigated system is more thermodynamically favoured.

[6] A few 'pseudo'-18-norsteroids, which have the 18-methyl group migrated to C-17, have been reported. J. TOMKO, A. VASSOVA, G. ADAM, K. SCHREIBER and E. HÖHNE, Tetrahedron Letters *1967*, 3907.

Morphogenetic Effects of Follicle Stimulating Hormone: Effects on the Synthesis of Nuclear RNA's by Chick Embryos

The processes of development, growth and maintenance of an organism are directed, in some phase or other, by hormones. Hormones are involved, for instance, in the processes of differentiation of germ cells[1] and mammary tissue[2,3], growth and moulting of insects[4,5], and metamorphosis in amphibia[6].

Follicle-stimulating hormone (FSH) is a glycoprotein hormone produced by the anterior pituitary gland. It is responsible for the growth and maturation of ovarian follicles. The possibility that it might get localized in the developing ovum and also play a role in organizing embryogenesis was suggested several years ago[7]. It was later shown to be able to induce differentiation of neural, notochordal, and somitic tissues in chick embryonic systems[8-10]. Cellular differentiation is a phenotypic expression of differential genetic activity. The morphogenetic property of the hormone probably reflects its ability to control genetic activity. If it were so it might produce specific effects on RNA synthesis by developing embryos.

Material and methods. Chick embryos at stage 4 (medium primitive-streak), stage 5 (head-process), stage 7 (neural-fold embryos with 1–2 pairs of somites), and stage 9 (embryos with 8–9 pairs of somites) were given H^3-uridine (25 μc/ml) in 15 min, 1 and 3 h pulses. Some embryos of each stage were treated with 0.5 mg/ml FSH (NIH-FSH-S4) for 1 h before the precursor was supplied. All of them were sectioned and autoradiographed after extraction with 5% TCA at 4°C. The experiments were controlled using RNase. Nuclear grain counts were made in epiblast, mesoderm or their derivative tissues, viz. the neural tissue, notochord and somites respectively. The data are presented in Tables I and II. H^3-butylmethacrylate (50 μc/g from Radiochemical Centre, Amersham) sections were used as standards and autoradiographic efficiency (ARE) was determined for each batch of autoradiographs[11].

Results and discussion. The object of these experiments was to see if the hormone produced any differential effect on the synthesis of nuclear RNA's (nRNA) by the different embryonic tissues and if they did, whether these effects could be correlated with morphogenetic events occurring at the time of treatment with the hormone. Differential effects were indeed noticed, and these were also found to be stage-specific.

Mesoderm cells appeared to have been stimulated by the hormone (see Table I) beginning with stage 5. The synthesis of nRNA by epiblast and mesoderm cells of stage 4 and epiblast cells of stage 5 was found to be inhibited by the hormone. But mesodermal cells of stage 5 embryos did not show such an inhibition. This may be related to the fact that this is roughly the stage of development when mesoderm enters into various inductive interactions. This is also the stage which is highly susceptible to anti-FSH action[9]. We do not know how an inhibition of nRNA synthesis in stage 4 cells might have

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[1] ET. WOLFF, in *Cell Differentiation*, CIBA Foundation Symposium (Ed. A. V. S. DE REUCK and J. KNIGHT; J. A. Churchill, London 1967), p. 143.

[2] F. E. STOCKDALE, W. G. JUERGENS and Y. J. TOPPER, Devl. Biol. *13*, 266 (1966).

[3] D. H. LOCKWOOD, F. E. STOCKDALE and Y. J. TOPPER, Science *156*, 945 (1967).

[4] W. J. BURDETTE, Cancer Res. *24*, 521 (1964).

[5] K. C. HIGHNAM, J. Endrocrin. *39*, 123 (1967).

[6] J. R. TATA, Progr. nucl. Acid Res. molec. Biol. *5*, 191 (1966).

[7] G. V. SHERBET, Naturwissenschaften *49*, 471 (1962); J. Embryol. exp. Morph. *11*, 227 (1963).

[8] G. V. SHERBET and L. MULHERKAR, Wilhelm Roux Archiv. EntwMech. *154*, 506 (1963); *155*, 701 (1965).

[9] G. V. SHERBET and M. S. LAKSHMI, Experientia *23*, 969 (1967); Nature *217*, 1257 (1968).

[10] G. V. SHERBET and M. S. LAKSHMI, Nature *215*, 1089 (1967); Experientia, *25*, 481 (1969).

[11] ARE is defined as the number of silver grains formed per 100 disintegrations occurring in the standard section of infinite thickness with reference to tritium (i.e. not less than 5 μ).