This assumption is supported by the fact that the observed coupling constant of the proton at carbon-3 is larger than that of other protons in ESR spectrum of the free radical produced from I,[7] and the final step, deoxygenation, would take place similarly by a free radical course as in the case of deoxygenation of 4-nitroquinoline 1-oxide.[8] The formation of 3,3'-biquinoline compounds and deoxygenation of N-oxide were also recognized in the reaction of 4-nitroquinoline 1-oxide and 4-hydroxyquinoline 1-oxide, indicating that a free radical would be present as an intermediate. Further studies are in progress on the chemistry of 4-hydroxyaminoquinoline 1-oxide and 4-nitroquinoline 1-oxide, in view of the free radical reactivity in relation to their carcinogenic activity.

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UDC 581.19 : 547.92.02 : 582.675.1

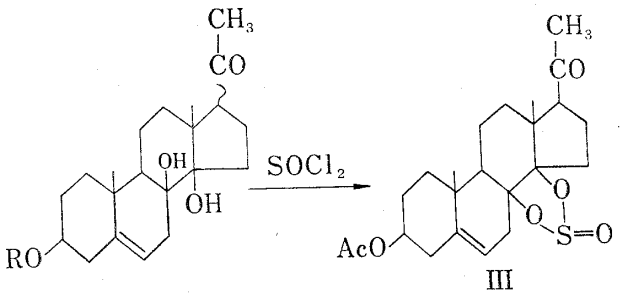
Isolation and Structures of New Pregnane Derivatives from *Adonis amurensis* REGEL et RADD

In a previous communication,[1] we reported the isolation and the structure of a non-cardiac aglycone, adonilide, from *Adonis amurensis* REGEL et RADD. In this communication, we wish to describe the isolation and the structures of three novel non-cardiac aglycones: fukujusone (I), ester A (V) and ester B (VI).

Fukujusone (I), mp 224—227°, $[\alpha]_D$ +100.0° (c=1.0, $CHCl_3$) has a formula $C_{21}H_{32}O_4$ (*Anal.* Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.61, H, 9.07. molecular peak: m/e 348). The infrared (IR) spectrum (ν_{max}^{Nujol} cm^{-1}: 3550, 3529, 1680) demonstrates the presence of hydroxyl groups and a carbonyl function. The NMR spectrum shows the following signals: τ (in $CDCl_3$) 8.80 (3H, singlet, CH_3), 8.78 (3H, singlet, CH_3) 7.72 (3H, singlet, $-COCH_3$), 7.10 (1H, multiplet), 6.50 (1H, broad multiplet, C<u>H</u>–OH), 4.60 (1H, multiplet, vinylic proton). Acetylation of I with acetic anhydride in pyridine afforded a monoacetate (II), mp 196—198°, $[\alpha]_D$+61.5° (c=1.39, $CHCl_3$), (*Anal.* Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.98; H, 8.95). The IR spectrum (ν_{max}^{Nujol} cm^{-1}: 35600, 3400, 1720, 1680) shows the presence of hydroxyl groups which are not acetylable under this condition. The nuclear magnetic resonance (NMR) spectrum of II has the following signals: τ (in $CDCl_3$) 8.80 (3H, singlet, CH_3), 8.72 (3H, singlet, CH_3), 7.90 (3H, singlet, $-COCH_3$), 7.70 (3H, singlet, $-COCH_3$), 7.10 (1H, multiplet, –C<u>H</u>–CO–), 5.30 (1H, broad multiplet, C<u>H</u> –OAc), 4.50 (1H, multiplet, vinylic proton). From the above data, we concluded that fukujusone (I) has a secondary hydroxyl group, a methyl ketone, two angular methyl groups, and a vinylic proton. Considering the structural simil-

1) Y. Shimizu, Y. Sato and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **15**, 2005 (1967).

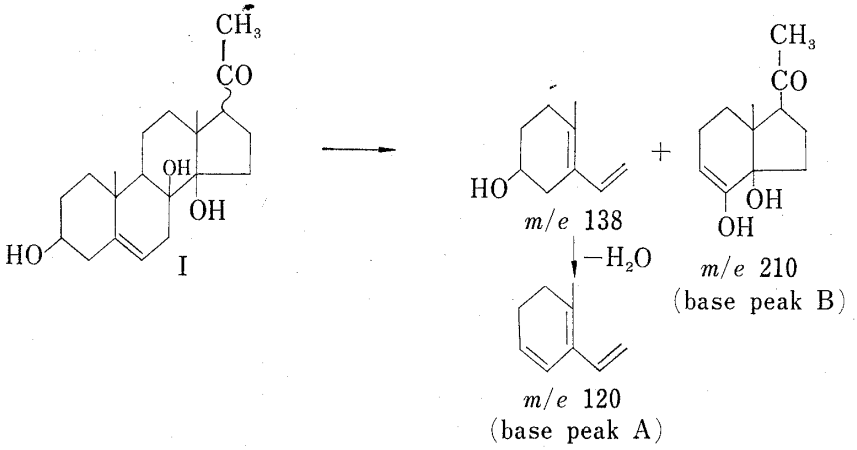
arities expected from the biogenetic view point, it was assumed that fukujusone (I) also comprises a partial structure of 3β-hydroxy-5-pregnen-20-one, as do the other isolated compounds. This was supported by the splitting pattern of signals at τ 6.50 and 4.60 characteristic of 5-en-3β-ol, and the mass spectrum data (*vide infra*). The absence of other signals in the τ 4—6 region of the NMR spectrum suggests that the remaining two oxygens exist as tertiary hydroxyl groups. The presence of 17-hydroxyl group was excluded, because the multiplet at τ 7.10 was considered to be the C-17-methine proton, as exemplified later by the formation of the 17-*iso*-derivative. Acetylfukujusone (II) consumed one mole of lead tetraacetate, indicating the tertiary alcohols exist as an α-glycol. And the fact that both angular methyl groups are considerably deshielded made us assign one of the alcohols to 8β-position.[2] Accordingly, the other one should be located at either C-9 or C-14 position. The 8β,14β-glycol is known to form a cyclic sulphite with $SOCl_2$ rather than to undergo dehydration.[3] Thus treatment of (II) with $SOCl_2$ in pyridine gave a five-membered cyclic sulphite, (III), mp 195—198°. IR ν_{max}^{Nujol} cm^{-1}: 1213—1215 $\left(\begin{matrix}O\\O\end{matrix}\!\!>\!S=O\right)$. The remaining problem is the stereochemistry at C-17, and it was establisehd by the following evidence.



III

I : R=H, 17β
II : R=Ac, 17β
IV : R=H, 17α

Being treated with a solution of 5% methanolic potassium hydroxide, (I) isomerized predominantly to isofukujusone (IV), mp 212—217°, $[\alpha]_D$ −31.0° (c=1.08, $CHCl_3$) (*Anal.* Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.48; H, 9.52. molecular peak at: m/e 348); ν_{max}^{Nujol} cm^{-1}: 3600, 3590, 3580, 1680: τ (in $CDCl_3$) 8.84 (3H, singlet, CH_3), 8.64 (3H, singlet, CH_3), 7.86 (3H, singlet, $-COCH_3$), 6.72 (1H, multiplet, $-CHCO-$), 6.50 (1H, broad multiplet, $\underline{C}H-OH$), 4.68 (1H, multiplet, vinylic



m/e 330(M–H_2O), 315(M–H_2O–CH_3), 312(M–$2H_2O$), 297(M–$2H_2O$–CH_3), 287(M–H_2O–CH_3CO), 279(M–$3H_2O$–CH_3), 269(M–$2H_2O$–CH_3CO), 251(M–$3H_2O$–CH_3CO), 192(B–H_2O), 177(B–H_2O–CH_3), 149(B–H_2O–CH_3CO), 134(B–H_2O–CH_3–CH_3CO), 123(B–$2H_2O$–CH_3–CH_3CO), 105(A–CH_3).

Chart 1. Mass Fragmentation of Fukujusone (I)

2) *a*) K. Tori and E. Kondo, *Tetrahedron Letters*, **1963**, 645; *b*) Y. Shimizu and H. Mitsuhashi, *Tetrahedron*, **24**, 4143 (1968).

3) A. von Wartburg and J. Renz, *Helv. Chim. Acta*, **42**, 1639 (1959); A. von Wartburg and J. Renz, *ibid.*, **42**, 1646 (1959); R. Tschesche and G. Marwede, *Tetrahedron Letters*, **1967**, 1359.

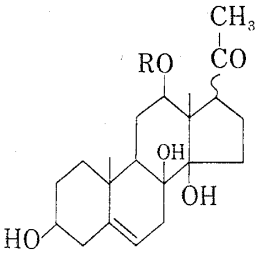
proton). The optical rotatory dispersion (ORD) curve of IV shows a negative Cotton effect (trough $[\phi]_{309}$ −2800°, peak $[\phi]_{268}$ +4240°, in MeOH), while the ORD curve of I possesses a maximum peak at 304 mμ ($[\phi]_{304}$ +3170°) with the trough covered under the strong positive background. This relation between the ORD sign and equilibration is that of 14β-pregnan-20-one with C/D-*cis* ring juncture, where the 17α-orientation is more stable.[4]

The mass spectrum data of I and IV are very similar and fully compatible with the structures. The base peaks, *m/e* 120, *m/e* 210 and the other prominent peaks could be accounted for by the following scheme (Chart 1).

Thus, we believe that fukujusone has the structure of 3β,8β,14β-trihydroxy pregn-5-en-20-one. It is of some biogenetic interest that fukujusone corresponds to the 12-desoxy derivative of isolineolon which was also found in the same plant.

Ester A (V), mp 250—254°, $[\alpha]_D$ +46° (c=1.26, $CHCl_3$) has a formula $C_{27}H_{35}O_6N$ (*Anal.* Calcd. for $C_{27}H_{35}O_6N$: C, 69.06; H, 7.51, N, 2.98. Found: C, 69.53; H, 7.87, N, 3.24). The ultraviolet (UV) spectrum of V has an absorption maximum at λ_{max}^{EtOH} 264 mμ (log ε 3.60). From the IR spectrum (ν_{max}^{Nujol} cm^{-1}: 3600, 3500, 3459, 1680, 1720, 1595), the presence of hydroxyl, carbonyl, ester, and aromatic groups was anticipated. The NMR spectrum exhibits the following signals: τ (in $CDCl_3$) 8.75 (3H, singlet, CH_3), 8.60 (3H, singlet, CH_3), 7.76 (3H, singlet, CH_3), 6.76 (1H, multiplet, –C<u>H</u>CO–), 6.50 (1H, broad multiplet, C<u>H</u>–OH), 5.00 (1H, quartet, CH–O–CO–), 4.60 (1H, multiplet, vinylic proton), 1.60—2.50 (4H, aromatic proton).

Hydrolysis of (V) with 5% methanolic potassium hydroxide gave an amino acid, which was separated by ion-exchange resin and identified as nicotinic acid. The neutral fraction was a mixture of two substances, lineolon (VII) and isolineolon (VIII), which were identified with the samples isolated from *Cynanchum caudatum*.[2b] Therefore, ester A (V) should be formulated as 12-nicotinoyllineolon or -isolineolon. The location of the ester linkage was deduced by the splitting pattern of the hydrogen adjacent to the esterified hydroxyl group (quartet, J=5 and 11 cps).[2b] The authors believe that the ester A is the first example of a plant steroid conjugated with an amino acid.



V : R=  –CO–, 17β

VI : R=Phenyl–CO–, 17β

VII : R=H, 17α

VIII : R=H, 17β

The other ester aglycone, ester B (VI), mp 245—250°, $[\alpha]_D$ +45° (c=0.82, $CHCl_3$) has a formula, $C_{28}H_{36}O_6 \cdot 1/2H_2O$ (*Anal.* Calcd. for $C_{28}H_{36}O_6 \cdot 1/2H_2O$: C, 70.44 H, 7.80. Found: C, 71.06 H, 7.80). The UV spectrum of VI has an absorption maximum at λ_{max}^{EtOH} 233 mμ (log ε 4.10), 278 mμ (log ε 3.13). The IR spectrum (ν_{max}^{Nujol} cm^{-1}: 3620, 3600, 3500, 1720, 1690, 1590, 1270) shows the presence of hydroxyl, carbonyl, ester and aromatic groups. The hydrolysis of VI with 5% methanolic potassium hydroxide gave an acidic substance, which was identified with benzoic acid. The neutral fraction consisted of lineolon (VII) and isolineolon (VIII). Thus VI is a monobenzoyl ester of lineolon or isolineolon. However, ester B (VI) is different from the substance *G* reported by Abisch, *et al.*[5] but identical with the sample isolated from *Cynanchum boerhavifolium*, and its structure will be published in a different paper.[6] In addition, free lineolon (VII) and isolineolon (VIII) were isolated from the polar fraction of the aglycone mixture.

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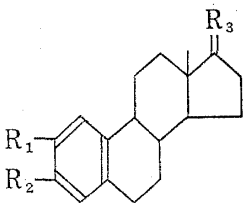
Occurrence of "NIH Shift" during Hydroxylation of Aromatic Steroid

It has recently been discovered that during enzymatic hydroxylation of aromatic substrates the substituent (^{2}H, ^{3}H, Cl, Br, *etc.*) displaced by the entering hydroxyl group migrates to an adjacent position. These phenomena called "NIH shift" have been demonstrated with enzymes derived from animal and plant sources.[1,2] We have previously reported that hydroxylation does take place at C-2 and C-3 when 3-deoxyestrone (I) is orally given to rabbit.[3] Therefore it seemed to be of considerable interest to us to elucidate whether the aromatic steroid would similarly undergo "NIH shift" or not. We now wish to report the *in vivo* hydroxylation of the specifically deuterated 3-deoxyestrone.



I : $R_1=R_2=H$, $R_3=O$
II : $R_1=OH$, $R_2=H$, $R_3=\overset{OH}{H}$
III : R_1=1-phenyl-5-tetrazolyloxy-, $R_2=H$, $R_3=\overset{OH}{H}$
IV : $R_1=D$, $R_2=H$, $R_3=O$
V : $R_1=H$, $R_2=OH$, $R_3=O$
VI : $R_1=H$, R_2=1-phenyl-5-tetrazolyloxy-, $R_3=O$
VII : $R_1=H$, $R_2=D$, $R_3=O$

First, 2-deuteriosteroid was prepared as a substrate from 2-hydroxy-3-deoxyestradiol (II) in three steps. Condensation of II with 1-phenyl-5-chlorotetrazole in the presence of potassium carbonate[4] gave the 2-(1-phenyl-5-tetrazolyl) ether (III), mp 140—141°, as colorless needles (from aq. acetone). Catalytic reduction over palladium–on–barium carbonate under a stream of deuterium gas followed by oxidation with Jones reagent furnished the desired 2-deuterio-3-deoxyestrone (IV), mp 141—142°, as colorless needles (from ether). Likewise 3-deuterio-3-deoxyestrone (VII) was also synthesized starting from estrone (V) by way of 3-(1-phenyl-5-tetrazolyl) ether (VI), mp 204—206° (from aq. acetone). The distribution and quantity of the isotope in these selectively labelled steroids were determined by means of nuclear magnetic resonance and mass spectrometries.

A single dose of suspension of 2-deuterio-3-deoxyestrone (IV) (475 mg) in Tween 80 was orally given to a male rabbit weighing about 2.3 kg. The urine was collected for the following

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