CHEMICAL
&
PHARMACEUTICAL BULLETIN

Vol. 12 No. 7 July 1964

[Chem. Pharm. Bull. 12 (7) 755 ~ 760]

UDC 582.998 : 581.19

105. Hiroshi Hikino, Yasuko Hikino,*1 and Itiro Yosioka*2 : Studies on the Constituents of Atractylodes. IX.*3 Structure and Autoxidation of Atractylon.

(*Pharmaceutical Institute, School of Medicine, Tohoku University*,*1 *and Faculty of Pharmaceutical Sciences, Osaka University*,*2)

During the chemical investigations of the constituents of the crude drug "Jutsu," Takagi[1,2] obtained two crystalline substances, to which he gave the names atractylol and atractylon, from the rhizomes of what he considered to be *Atractylis ovata* THUNBERG. Recent research[3,4] has shown that atractylol is a mixture of hinesol and eudesmol and that the crude drug from which so-called atractylol was obtained probably originated from *Atractylodes lancea* DE CANDOLLE. More recently the authors have obtained a compound whose properties correspond to those reported for atractylon, from the crude drugs known as "Tohoku-dai-ko-jutsu,"[5] "Wa-so-jutsu," and "Kan-byaku-jutsu."[6] The first of these drugs is prepared from the rhizome of Manchurian Atractylodes plant which is considered to be related histologically to those of *Atractylodes japonica* KOIDZUMI and *A. chinensis* KOIDZUMI.[7] The latter two are obtained from the rhizomes of *A. japonica*, of Japanese and Korean origin, respectively.

As part of the chemical investigation of Atractylodes plants, the authors have studied the chemistry of atractylon and, in a preliminary communication,[8] assigned the structures (I, IV, and V) to atractylon and its two autoxidation products, respectively. The present paper describes the evidence in full detail.

The earlier work[2] on the chemistry of atractylon indicated the formula $C_{14}H_{20}O$ and that the compound was probably a tricyclic oxide containing three unconjugated ethylenic linkages. At the outset of this work, it was found that atractylon melted at 38° and had the composition $C_{15}H_{20}O$. The infrared absorption spectrum exhibited no band associated with an oxygen function, such as hydroxyl or carbonyl group, but a band at 1134 cm^{-1} which suggested the presence of an oxide function in the molecule. The presence of a trisubstituted furan system, which was not clear by the infrared absorption, was determined from the nuclear magnetic resonance spectrum of atrac-

*1 Kita-4-bancho, Sendai (ヒキノ ヒロシ, 曳野靖子).
*2 Toneyama, Toyonaka, Osaka (吉岡一郎).
*3 Part Ⅷ. I. Yosioka, H. Hikino, Y. Sasaki : This Bulletin, 8, 957 (1960).
1) S. Takagi : Yakugaku Zasshi, No. 473, 565 (1921).
2) S. Takagi, G. Hongo : *Ibid.*, No. 509, 539 (1925).
3) S. Takahashi, H. Hikino, Y. Sasaki : *Ibid.*, **79**, 544 (1959).
4) I. Yosioka, S. Takahashi, H. Hikino, Y. Sasaki : This Bulletin, **7**, 319 (1959).
5) *Idem* : Yakugaku Zasshi, **80**, 1564 (1960).
6) I. Yosioka, H. Hikino, Y. Hikino : unpublished data.
7) S. Takahashi, K. Namba : Shoyakugaku Zasshi, **15**, 246 (1961).
8) H. Hikino, Y. Hikino, I. Yosioka : This Bulletin, **10**, 641 (1962).

tylon; an unresolved band at 3.15 τ was attributed to the α-hydrogen, while a doublet at 8.15 τ (J=1 c.p.s.) corresponded to the protons of the β-methyl group. This evidence was further supported by positive color tests for furan ring such as vanillin-hydrochloric acid, pine stick, Ehrlich, and Liebermann-Burchard reactions, by the ultraviolet absorption maximum at 220 mμ, and by formation of a Diels-Alder adduct with maleic anhydride. Confirmation of the exocyclic nature of the third ethylenic linkage in atractylon was furnished by infrared absorption bands at 3077, 1639, and 886 cm^{-1}, by the nuclear magnetic resonance spectrum which showed two peaks at 5.26 and 5.39 τ, and by the formation of formaldehyde on ozonolysis. Catalytic hydrogenation in methanol over Adams' catalyst resulted in the addition of 1 mole of hydrogen to the vinylidene system to give dihydroatractylon (II). The retention of the furan ring was indicated by the nuclear magnetic resonance spectrum which still showed the bands at 3.15 τ and 8.20 τ, by positive reactions for the previous color tests, and by the ultraviolet maximum at 221 mμ. No conclusion could be reached as to the existence of the furan system from the infrared spectrum of the dihydroderivative (II), which, however, showed a remarkable similarity with the spectrum of menthofuran. Catalytic hydrogenation of atractylon in ethyl acetate using a palladized-charcoal catalyst led to the absorption of three moles of hydrogen to yield the hexahydro-derivative. With the aid of gas chromatography it was shown that this saturated product consisted of two components which were considered to be stereoisomeric. On crystallization the major component (III) was identified as the major component of octahydrodehydroxylinderene, the reduction product of linderene.[9] Recently the stereochemistry of the oxide (III) has been established by its correlation with the known tetrahydroalantolactone (X).[10] Hence atractylon is elucidated as shown in formula (I).

It has been pointed out by Takagi[2] that atractylon is readily resinified on standing in air. On solution in organic solvents, it is gradually autoxidized, leading ultimately to two crystalline compounds, the autoxidation product A, $C_{15}H_{20}O_2$, m.p. 125°, and B, $C_{15}H_{20}O_3$, m.p. 197~197.5°. These products have also been isolated from the original essential oil of *A. japonica*.[6]

The infrared spectrum of each of the autoxidation products A and B exhibited bands due to an α,β-unsaturated γ-lactonic carbonyl group at 1733 and 1736 cm^{-1}, respectively, and to an ethylenic linkage conjugated with the lactonic carbonyl group at 1672 and 1695 cm^{-1}, respectively. The ultraviolet maximum at 220 mμ also supported the presence of the α,β-buthenolide system. Both compounds gave a yellow color with tetranitromethane and showed infrared absorption at 900 and 897 cm^{-1}, respectively, characteristic of a vinylidene group. This was in accordance with the isolation of formaldehyde on ozonolysis. Catalytic hydrogenation of both products under moderate conditions resulted in the saturation of the exocyclic double bond to afford the dihydroderivatives, (VI) and (VII), respectively, which no longer had the infrared absorption attributed to the vinylidene feature but still had maxima associated with the α,β-butenolide system.

The third oxygen function in the autoxidation product B was shown to be a hydroxyl group by the infrared band at 3333 cm^{-1}. In addition, that the hydroxyl group was located in a part of a hemiketal lactone form was established from the following facts. (1) The product B is weakly acidic, shown by its solubility in sodium hydrogen carbonate solution. (2) Though the ultraviolet absorption in neutral solution disclosed no maximum except that at 220 mμ, the spectrum of the product B in ethanolic sodium hydroxide solution displayed a maximum at 264 mμ which can be ascribed to a γ-oxo-

9) T. Takeda, T. Shimada : Yakugaku Zasshi, **64**, 154 (1944).
10) K. Takeda, M. Ikuta : Tetrahedron Letters, **1964**, 277.

α,β-unsaturated carboxylate ion. (3) On reaction with phenylhydrazine in ethanol, the product B gave a crystalline compound, whose analysis corresponded to the expected formula (Ⅷ). (4) Dehydrogenation by heating with potassium hydrogen sulfate converted the product B into an oily anhydro-derivative whose infrared bands at 1770, 1673, and 1647 cm^{-1} and the ultraviolet maximum at 275 mμ were taken as characteristics of an α,β-butenolide with extended conjugation. However, the infrared absorption no longer indicated the presence of the vinylidene function, as a result of the migration of the exocyclic double bond to an endocyclic position under such drastic conditions. Dehydration of the product B with phosphorus oxychloride in pyridine afforded a crystalline anhydro-derivative (Ⅸ) whose infrared spectrum also showed bands at 1765, 1665, and 1649 cm^{-1} associated with the α,β-butenolide with extended conjugation and band at 895 cm^{-1} indicating the retention of the vinylidene group. The autoxidation product B was regenerated from the anhydro-compound (Ⅸ) by dissolving it in aqueous alkali, followed by acidification.

Hydrogenation of the product B over Adams' catalyst in acetic acid in the presence of a minute amount of hydrogen chloride involved elimination of the hydroxyl group to give a saturated lactone which was found to be identical with tetrahydroalantolactone (Ⅹ).[11] Arranging the above functions on this skeleton gives formula (Ⅴ) as the constitution for the autoxidation product B, except the configuration at C-8 which is discussed below.

The molecular formula and the spectral properties suggest that the product A has structure (Ⅳ). This was confirmed by identifying the dihydro-derivative with the butenolide (Ⅵ) derived from alantolactone.[12] Since Tanabe[13] has shown that reduction of the lactone (Ⅵ) gives the saturated lactone of stereoformula (Ⅺ), the orientation of the C-8 hydrogen in the product A is therefore β.

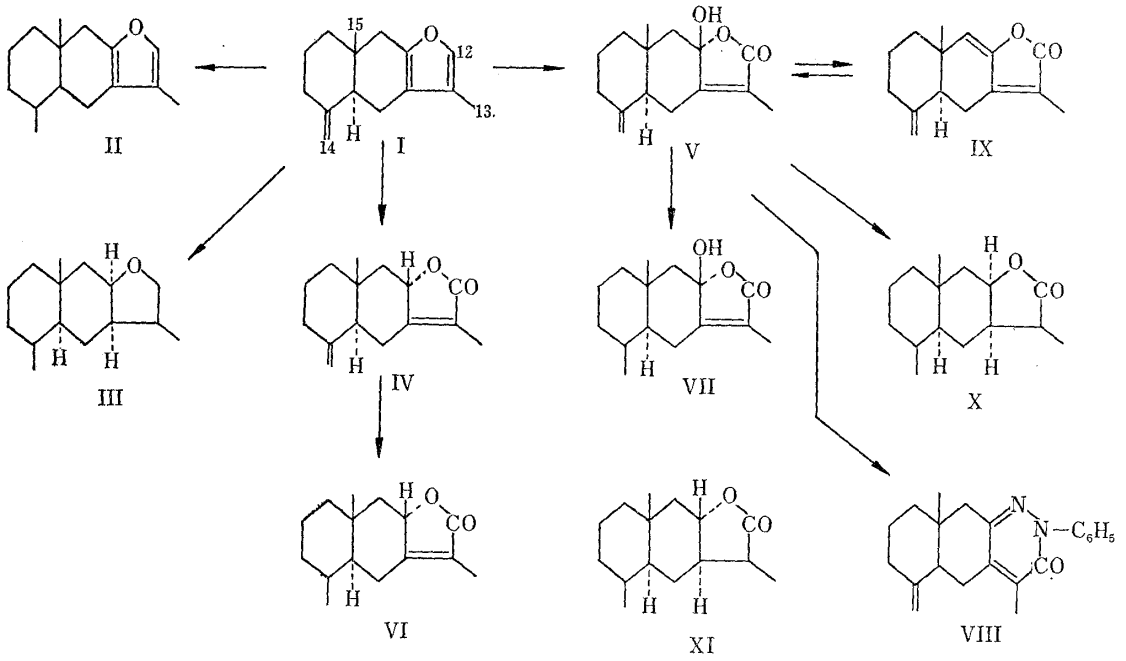
The configuration at C-8 of the product B was deduced as follows: if the C-8 hydroxyl group is α-oriented, the fusion with the butenolide ring requires the cyclohexane ring to be in a twist-boat conformation. It was found that the product B was readily regenerated by acid from its solution in alkali. This indicates that the product B has the more stable conformation (*i.e.* β-hydroxyl) at C-8. This assignment was also examined by the nuclear magnetic resonance spectra; thus, the C-15 methyl protons of the product A (Ⅳ) appeared at 9.10 τ, while those of the product B (Ⅴ) occurred at 8.96 τ, a down-field shift of 0.14 p.p.m. in agreement with a 1,3-diaxial relation[14] between the C-15 angular methyl group and the C-8 hydroxyl group in the product B. Therefore it was concluded that the C-8 hydroxyl group in the product B was in the β-configuration.

It is possible to account for all the other peaks in the nuclear magnetic resonance spectra on the basis of the structures (Ⅳ and Ⅴ) for the autoxidation products A and B, respectively. (See experimental part).

The autoxidation process responsible for the conversion of atractylon into the product B has been previously observed;[15] however, reactions similar to the formation of the product A do not seem to have been described. Recently, Novotný, *et al.*[16] have

11) W. Cocker, M. A. Nisbet: J. Chem. Soc., **1963**, 534.
12) H. Matsumura, I. Iwai, E. Ohki: Yakugaku Zasshi, **74**, 1029 (1954).
13) K. Tanabe: personal communication.
14) Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto, K. Tsuda: This Bulletin, **10**, 338 (1962).
15) G. O. Schenck: Ber., **77**, 661 (1944); *Idem*: Angew. Chem., **60A**, 224 (1948); R. B. Woodward, R. H. Eastman: J. Am. Chem. Soc., **72**, 399 (1950); A. Melera, K. Schaffner, D. Arigoni, O. Jeger: Helv, Chim. Acta, **40**, 1420 (1957).
16) L. Novotný, J. Jizba, V. Herout, F. Šorm: Collection Czechoslov. Chem. Commums., **27**, 1393 (1962); J. Hochmannová, L. Novotný, V. Herout: *Ibid.*, **27**, 1870 (1962).

isolated furanoeremophilane and eremophilenolide from *Petasites officinalis* MOENCH. The latter may have been derived from the former by an autoxidation process similar to that of the conversion of atractylon to the product A.



Experimental[*4]

Atractylon——$C_{15}H_{20}O$, m.p. 38°, $[\alpha]_D$ +40.0° (c=10.0), UV : λ_{max}^{EtOH} 220 mμ (log ε 3.89), IR (Nujol) cm^{-1} : 1134 (ether), 3077, 1639, 886 (vinylidene), NMR (at 40 Mc., in CCl_4 solution, H_2O as external reference calculated to be 5.30 τ) : described previously, vanillin-HCl reaction : + (scarlet), pine-stick test : + (violet), Ehrlich reaction : + (purple), Liebermann-Burchard reaction : + (scarlet).

The material (0.15 g.) was dissolved in anhyd. Et_2O (4 ml.) and a great excess (0.10 g.) of maleic anhydride was added. After 2 days the solution deposited an adduct which on crystallization from Et_2O yielded colorless needles, m.p. 118°, *Anal.* Calcd. for $C_{19}H_{22}O_4$: C, 72.59; H, 7.05. Found : C, 72.39; H, 7.03.

Ozonolysis of Atractylon—— Atractylon (1.0 g.) in AcOEt (20 ml.) was ozonized. The reaction mixture, decomposed by refluxing with H_2O and Zn dust, was steam-distilled. The distillate afforded formaldehyde 2,4-dinitrophenylhydrazone, m.p. 161~162°, *Anal.* Calcd. for $C_7H_6O_4N_4$: N, 26.66. Found : N, 26.71, undepressed on admixture with an authentic sample.

Partial Hydrogenation of Atractylon——Atractylon (1.0 g.) in MeOH (60 ml.) with PtO_2 (0.03g.) was hydrogenated. The uptake of H_2 ceased after 1 mole had been consumed. The product was employed in the usual way to give dihydroatractylon (II) as a colorless mobile oil, $b.p_3$ 101°, n_D^{25} 1.513, α_D −44.0°, *Anal.* Calcd. for $C_{15}H_{22}O$: C, 82.51; H, 10.16. Found : C, 83.06; H, 10.04, UV : λ_{max}^{EtOH} 221 mμ (log ε 3.82), IR (liquid film) : 1139 cm^{-1} (ether), NMR (at 40 Mc., in CCl_4 solution, H_2O as external reference calculated to be 5.30 τ) : singlet at 9.15 τ (C-15 methyl), doublet at 9.10 τ (J=10 c.p.s.; C-14 methyl), doublet at 8.20 τ (J=1 c. p. s. ; C−13 methyl), unresolved band at 3.15 τ (C-12 hydrogen), vanillin-HCl reaction : + (scarlet), pine-stick test : + (violet), Ehrlich reaction : + (purple), Liebermann-Burchard reaction : + (scarlet).

Complete Hydrogenation of Atractylon——Atractylon (6.0 g.) in MeOH (100 ml.), when stirred with Pd-C (5%; 7.0 g.), absorbed 3 moles of H_2. Working up in the usual manner and chromatography in light petroleum solution over Al_2O_3 afforded the hexahydro-derivative as a colorless mobile oil, $b.p_6$ 120 ~122°, d_4^{25} 0.979, n_D^{25} 1.496, α_D −51.4°, *Anal.* Calcd. for $C_{15}H_{26}O$: C, 81.02; H, 11.79. Found : C, 81. 30; H, 11.42.

By means of gas chromatography,[*5] the oil was found to consist of two constituent of which the major component (III) crystallized from light petroleum to give colorless plates, m.p. 22~24°, IR (Nujol) : 1037 cm^{-1} (ether). The IR spectrum of this compound was identified with that of the major component of octahydrodeoxylinderene.

[*4] All melting points and boiling points are uncorrected. $[\alpha]_D$s refer to $CHCl_3$ solutions.

[*5] 10% SE30-fire brick, 0.5×200 cm., 200°, He 30 ml./min.

Autoxidation of Atractylon——A solution of atractylon (1.0 g.) in MeOH (20 ml.) was left standing at room temperature in the presence of air for 20 days. After evaporation of the solvent, the product was dissolved in Et_2O and extracted with Na_2CO_3 solution.

The ethereal solution was washed with H_2O, dried (Na_2SO_4), and evaporated; crystallization from EtOH afforded atractylon autoxidation product A (Ⅳ) as colorless needles, m.p. 125°, $[\alpha]_D$ +266.1° (c=5.0), *Anal.* Calcd. for $C_{15}H_{20}O_2$: C, 77.54; H, 8.68. Found: C, 77.20; H, 8.58, UV λ_{max}^{EtOH} mμ (log ε): 220 (4.21), 276 (1.41), UV: $\lambda_{max}^{NaOH-EtOH}$ 231 mμ (log ε 3.74), IR (Nujol) cm^{-1}: 1733, 1672 (butenolide), 3067, 1639, 900 (vinylidene), NMR (at 60 Mc., in $CDCl_3$ solution, Me_4Si as internal reference): singlet at 9.10 τ (C-15 methyl), triplet at 8.18 τ (J=1.6 c.p.s.; C-13 methyl), two peaks at 5.39 and 5.15 τ (C-8 hydrogen and C-14 methylene).

The Na_2CO_3 solution was acidified with dil. HCl to deposit a solid which, on crystallization from EtOH, gave atractylon autoxidation product B (Ⅴ) as colorless needles, m.p. 197~197.5°, $[\alpha]_D$ +281.4° (c=5.0), *Anal.* Calcd. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.80; H, 8.16. UV: λ_{max}^{EtOH} 220 mμ (log ε 4.19), UV: $\lambda_{max}^{NaOH-EtOH}$ 264 mμ (log ε 3.73), IR (Nujol) cm^{-1}: 3333 (hydroxyl) 1736, 1695 (butenolide), 3070, 1637, 897 (vinylidene), NMR (at 60 Mc., in $CDCl_3$ solution, Me_4Si as internal reference): singlet at 8.96 τ (C-15 methyl), singlet with fine splitting at 8.21 τ (C-13 methyl), two peaks at 5.43 and 5.17 τ (C-14 methylene).

Ozonolysis of Atractylon Autoxidation Product A——Atractylon autoxidation product A (0.5 g.) in AcOEt (20 ml.) was ozonized at 0°. The ozonide was reduced by refluxing with H_2O and Zn dust, the volatile fraction was steam-distilled and the distillate gave formaldimedone as colorless needles (from EtOH), m.p. 186~187°, *Anal.* Calcd. for $C_{17}H_{24}O_4$: C, 69.83; H, 8.27. Found: C, 69.80; H, 7.99, undepressed in melting point on admixture with an authentic specimen.

Hydrogenation of Atractylon Autoxidation Product A——a) Atractylon autoxidation product A (1.0 g.) in AcOMe (50 ml.) was stirred with Pd-C (5%; 1.0 g.), when it absorbed 1 mole of H_2. The product was chromatographed on Al_2O_3 to give the dihydro-derivative (Ⅵ) as colorless needles, m.p. 114~115° (from EtOH), $[\alpha]_D$ +130.8° (c=6.4), *Anal.* Calcd. for $C_{15}H_{22}O_2$: C, 76.88; H, 9.46. Found: C, 76.66; H, 9.55, UV: λ_{max}^{EtOH} 221 mμ (log ε 3.80), IR (Nujol) cm^{-1}: 1735, 1678 (butenolide). The IR spectrum was identical with that of the authentic sample of the butenolide.

b) Atractylon autoxidation product A (0.10 g.) was hydrogenated over PtO_2 (0.10 g.) in AcOH (15 ml.) in the presence of conc. HCl (1 drop). One mole of H_2 was absorbed within 5 min., but no further uptake occurred. The product was worked up in the usual way and crystallized from light petroleum to afford the dihydro-derivative (Ⅵ) as colorless needles, m.p. 113~114°, identified with the dihydro-compound, above obtained, by the usual criteria.

Ozonolysis of Atractylon Autoxidation Product B——Atractylon autoxidation product B (0.1 g.) in AcOEt (10 ml.) was ozonized at 0°. The ozonide was decomposed by refluxing with H_2O and Zn dust and steam-distilled. The distillate formed formaldimedone, m.p. 187° (*Anal.* Calcd. for $C_{17}H_{24}O_4$: C, 69.83; H, 8.27. Found: C, 69.81; H, 8.17), which showed no depression of melting point on admixture with an authentic derivative.

Reaction of Atractylon Autoxidation Product B with Phenylhydrazine——Atractylon autoxidation product B (0.05 g.) was refluxed with phenylhydrazine (0.10 g.) in EtOH (2 ml.) for 24 hr. On dilution of the reaction mixture with H_2O, a viscous oil separated which gradually solidified. Crystallization from EtOH yielded yellow needles, m.p. 210~211°, *Anal.* Cacld. for $C_{21}H_{24}ON_2$: C, 78.71; H, 7.55; N, 8.74. Found: C, 78.62; H, 7.83; N, 9.07.

Hydrogenation of Atractylon Autoxidation Product B——Atractylon autoxidation product B (0.5 g.) in AcOEt (25 ml.) was stirred with Pd-C (5%; 0.5 g.), when it absorbed 1 mole of H_2 to afford the dihydro-derivative (Ⅶ) as colorless needles, m.p. 178~178.5° (from iso-Pr_2O), $[\alpha]_D$ +299.0° (c=4.1), *Anal.* Calcd. for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 72.14; H, 8.87, UV: λ_{max}^{EtOH} 221 mμ (log ε 4.10), IR (Nujol) cm^{-1}: 3356 (hydroxyl), 1730, 1695 (butenolide).

Hydrogenolysis of Atractylon Autoxidation Product B——Atractylon autoxidation product B (0.10 g.) in AcOH (10 ml.) in the presence of conc. HCl (1 drop) was shaken with PtO_2 (0.10 g.) in a H_2 atmosphere. Uptake of H_2 ceased after absorption of 3 moles. After filtration the mixture was made alkaline with NaOH solution and extracted with Et_2O. Evaporation of the dried ethereal extracts yielded no material. The alkaline solution was acidified with dil. H_2SO_4 and extracted with Et_2O. The ethereal layer was washed with H_2O, dried (Na_2SO_4), and evaporated. The residual oil (0.08 g.) crystallized from light petroleum giving the tetrahydrodeoxy-derivative (X) as colorless prisms, m.p. 141~143°, $[\alpha]_D$ +15.2° (c=10.0). The identity with tetrahydroalantolactone was established by the mixed melting point with an authentic sample and also identical IR spectrum.

Dehydration of Atractylon Autoxidation Product B with Potassium Hydrogen Sulfate——Atractylon autoxidation product B (0.10 g.) was heated with $KHSO_4$ (0.05 g.) at 180~190° at 5 mm. Hg. for 30 min. and distilled, yielding a viscous oil, b.p_5 190° (bath-temp.), *Anal.* Calcd. for $C_{15}H_{18}O_2$: C, 78.23; H, 7.88. Found: C, 78.31; H, 7.79, UV: λ_{max}^{EtOH} 276 mμ (log ε 4.25), IR (liquid film) cm^{-1}: 1770, 1673, 1647 (butadienolide), no vinylidene band.

Dehydration of Atractylon Autoxidation Product B with Phosphorus Oxychloride——$POCl_3$ (0.8 g.) was added dropwise with stirring to a solution of atractylon autoxidation product B (0.10 g.) in dry pyridine (2 ml.) at 0°. The mixture was set aside for 15 hr. at 0° and for a further 2 hr. at room temperature, poured on crushed ice, and extracted with Et_2O. The product was crystallized from EtOH to give the dehydrated product (Ⅸ) as colorless needles, m.p. 106~108°, *Anal.* Calcd. for $C_{15}H_{18}O_2$: C, 78.23; H, 7.88. Found: C, 78.44; H, 7.87, UV: λ_{max}^{EtOH} 275 mμ (log ε 4.32), IR (KBr) cm^{-1}: 1765, 1665, 1649 (butadienolide), 895 (vinylidene).

The dehydrated product (0.08 g.) was dissolved in *N* NaOH (5 ml.) on the steam-bath. Acidification with HCl deposited a precipitate which was crystallized from AcOEt giving the autoxidation product B (Ⅴ) as colorless needles, m.p. 193~195°. The identity was established by the usual criteria.

The authors are grateful to Dr. K. Takeda, Research Laboratory, Shionogi & Co., Ltd., who prepared a specimen of octahydrodeoxylinderene and to Drs. I. Iwai and K. Tanabe, Takamine Research Laboratory, Sankyo Co., Ltd., who provided specimens of tetrahydroalantolactone and the butenolide (Ⅵ) and gave them the valuable information about the stereochemistry of the butenolide (Ⅵ). Thanks are also due to Institute of Scientific and Industrial Research, Osaka University, and Research Laboratories, Takeda Chemical Industries, Ltd. for nuclear magnetic resonance spectra and to Analytical Laboratories, Faculty of Pharmaceutical Sciences, Osaka University, and Analytical Laboratories, Pharmaceutical Institute, School of Medicine, Tohoku University, for microanalyses and infrared spectra.

Summary

Atractylon, the sesquiterpenoid oxide isolated from the rhizomes of *Atractylodes japonica* and its related plants (Compositae), has been proven to have constitution Ⅰ on the basis of spectral properties and its conversion into 8,12-oxido-eudesmane. Autoxidation of atractylon has given two crystalline compounds, the autoxidation product A and B, which have been established as shown in formulae (Ⅳ and Ⅴ), respectively, from the degradative and spectral evidence.

(Received April 8, 1964)

[Chem. Pharm. Bull. 12 (7) 760 ~ 764] UDC 612.015[611.846.4]

106. Motosuke Kikutani and Kazuko Hirose: Studies on the Harderian Gland. III.*1 Purification and Properties of the Principle in Bovine Harderian Gland that increases the Serum Alkaline Phosphatase Activity.

(*Faculty of Pharmacy, University of Nagasaki**2)

It was previously reported that six protein fractions affecting serum alkaline phosphatase activity of rabbit were extracted and isolated from bovine Harderian gland.*1 Of these, fractions designated as A-40, CS-3, and CS-5*3 increased serum alkaline phosphatase activity and, threefore, each of them is considered to be the effective principle, because this enzyme activity level decreased markedly in a rabbit from which the Harderian gland was removed.[1]

---

*1 Part Ⅱ: Yakugaku Zasshi, **81**, 1154 (1961).

*2 Showa-machi, Nagasaki (菊谷元資, 広瀬和子).

*3 A-40=40% EtOH precipitated fraction; CS-3=30% $(NH_4)_2SO_4$ pptd. fraction; CS-5=50% $(NH_4)_2SO_4$ pptd. fraction.

1) M. Kikutani, Y. Takeuchi, Y. Nakamura: Yakugaku Zasshi, **80**, 1115 (1960).