same as above. Tests on mixtures of MMA, alkaline HIO_4, and aniline, and of MMA, HIO_3, and aniline were also carried out as above, using solutions (d) and (e). The results obtained are given in Table I.

Hydroxylation Test of Benzoic Acid—Following solution was prepared as a reagent besides (b), (c), and (d).

(f) 0.12 g. benzoic acid (guaranteed reagent) dissolved in 100 cc. of water.

In a Thumberg tube, 4 cc. of (b) diluted with 10 cc. of (f) was placed in the main tube, 6 cc. of (c) diluted with 10 cc. of (f) in a side tube, and the test was carried out as for the polymerisation test of MMA After 10 hr., the reaction mixture was filtered, the filtrate was made alkaline with Na_2CO_3, and extracted twice with 30 cc. of $CHCl_3$ to remove pigments. After making the aqueous solution acid with HCl, it was extracted with 20 cc. of Et_2O. The Et_2O layer was placed in a small test tube with 1 cc. of water, the solvent was evaporated, and $FeCl_3$ solution was added to the residual solution. The reaction mixture may be colored directly with $FeCl_3$ after adjusting its acidity to pH 2 with $HClO_4$, but adjustment of pH is rather complicated. Below pH 2, because of too high acidity, and above pH 2, because of a formation of ferric periodate, the reaction mixture does not color with $FeCl_3$ solution even if salicylic acid is present. Moreover, the oxidation products of aniline with HIO_4 contained a violet-colored substance and the coloration was not clear. Thus, the procedure with extraction mentioned above seemed to be more suitable.

From the mixtures of benzoic acid and HIO_4, of benzoic acid and HIO_3, and of aniline and HIO_4, no appearance of violet color was observed by the treatment mentioned above. These results are summarized in Table II.

Summary

Methyl methacrylate polymerised in a concentration of about $10^{-3}M$ in the reaction system of aniline oxidized with periodic acid, and benzoic acid was oxidized in a concentration of $10^{-2}M$ to salicylic acid in the same system. From these facts it was proved that the oxidation of aniline is a radical reaction and a free hydroxyl radical is produced, thereby proving the correctness of presumptions reported in the previous paper of this series.

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61. Itiro Yosioka, Shintaro Takahashi, Hiroshi Hikino, and Yasuko Sasaki: Studies on the Constituents of *Atractylodes.*[*1] III.[1] Separation of Atractylol into Eudesmol and Hinesol.

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It had been reported that a crystalline sesquiterpenoid alcohol, atractylol, had been isolated from the essential oil derived from the rhizomes of a certain *Atractylis* species (Compositae). Recently, the authors[1] determined that the original plant which gave the so-called atractylol was *Atractylodes lancea* DE CANDOLLE and not *Atractylis ovata* THUNBERG as had been believed, and that atractylol, which the earlier workers[2,3] had handled, might have been contaminated with other sesquiterpenoids and thus not completely pure.

Regarding previous works carried out on the chemical structure of this substance,

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1) Part II. S. Takahashi, H. Hikino, Y. Sasaki: Yakugaku Zasshi, **79**, 544(1959).
2) J. Gadamer, T. Amenomiya: Arch. Pharm., **241**, 22(1903).
3) S. Takagi: Yakugaku Zasshi, **473**, 565(1921).

it was first investigated by Ueno,[4] who considered it to have the composition $C_{10}H_{18}O$, but he was not able to obtain any characteristic product by various reactions. Gadamer[2] attempted to determine the structure of the alcohol having the composition $C_{15}H_{26}O$ and which is a tertiary alcohol shown by its difficulty to form esters and urethan, and also of the sesquiterpenoid hydrocarbon, atractylene, having two double bonds, prepared readily from the alcohol by dehydration, but they failed to make much progress in the elucidation of the structure. Chemical studies of atractylol were followed by Takagi[3] whose results may be briefly summarised as follows : Catalytic hydrogenation of atractylol over platinum in acid solution gave a liquid dihydroatractylol, while digestion of atractylol with formic acid resulted in dehydration to form the hydrocarbon, atractylene. The tetrahydro derivative of atractylene was identical with tetrahydromachilene. Oxidation of atractylol with permanganate yielded α-glycol, identical with dihydroxymachilol obtained by the same treatment of machilol, and β-glycol. These experiments led to a conclusion that atractylol (atractylene) has the same carbon skeleton as machilol (machilene) and differs from machilol only in the position of the ethylenic linkage and also of the tertiary hydroxyl group.

Later, Ruzicka[5] showed that machilol was identical with eudesmol by the comparison mainly of their dihydrochlorides and ozonolysis products, and suggested that atractylol was identical also with impure eudesmol contaminated by other sesquiterpenoid alcohol. In a later publication, Takagi[6] stated that Ruzicka's identification of atractylol with eudesmol had probably not been established by experimental evidence, but only through personal communication. Thus, there has not been any rigid proof of the structure of atractylol.

It was considered that atractylol, purified by repeated crystallisations from hydrous methanol in the present work, was in a higher state of purity than that described by earlier workers and looked as if it were a single substance, since it had a higher and sharp melting point and gave negative reactions in a few color test (i.e., Liebermann-Burchard test). Moreover, all specimens obtained from several lots of crude drug showed the same melting point, specific rotation, and infrared absorption spectrum. Nevertheless, the melting point and specific rotation of this substance were not identical with those of either the pure isomer or mixed crystals of eudesmols,[7] and it seemed possible that Ruzicka's suggestion might not have been right.

In order to elucidate the structure of atractylol, it was first submitted to various reactions,[1] regarding it as a single compound, and they seemed to proceed in the same manner as described by earlier investigators.[2,3] Reference has already been made to the experiments of Takagi[3] on the reduction of atractylol. It is well known that replacement of a hydroxyl group by hydrogen frequently occurs in a similar tertiary alcohol if the catalytic hydrogenation is carried out in the presence of platinum in acid medium, which was the condition selected by Takagi on the reduction of atractylol.

Then atractylol was hydrogenated over platinum in neutral medium which was considered to avoid the hydrogenolysis quoted above. Under such condition, it was found unexpectedly that hydrogen uptake ceased after absorption of only about half a mole of the theoretical value, calculated as $C_{15}H_{26}O$(〒) for this alcohol. The product exhibited a strong unsaturated character and on distillation afforded a colorless oil of almost constant boiling temperature, which later formed a crystalline paste, a mixture of colorless needles and an oil. It crystallised from acetone and was proved to be di-

4) K. Ueno : *Ibid.*, **129**, 1074(1892).
5) L. Ruzicka, D. R. Koolhaas, A. H. Wind : Helv. Chim. Acta, **14**, 1178(1931).
6) S. Takagi : " Asahina-Yasuhiko Hōbunshū, Chemical Part," 431(1934) (in note), Maruzen, Tokyo.
7) F. J. McQuillin, J. D. Parrack : J. Chem. Soc., **1956**, 2973.

hydroeudesmol. The oil separated from the crystals was distilled and gave a viscous substance, which seemed to be not yet completely pure but was considered to be a new sesquiterpenoid alcohol from its behavior. On catalytic hydrogenation over platinum in acid solution or over palladised charcoal in neutral solution, atractylol consumed 1 mole of hydrogen to give a saturated reduction product. These were evidences that atractylol must clearly consist of two different components, eudesmol, hydrogenation of which led to dihydroeudesmol, and a sesquiterpenoid alcohol of less reducible nature.

Then chromatographic examination of the material was tried to separate atractylol into two components. Chromatography of atractylol on alumina gave first, a crystalline material which was levorotatory. This was followed by materials of gradually increasing dextrorotation. The most strongly adsorbed material was found to be eudesmol.

Crystallisations of the first fraction, $[\alpha]_D$ −38.4 to−38.7°, formed a new sesquiterpenoid alcohol as stout white needles, m.p. 59~60°, $[\alpha]_D$ −40.2°, giving analysis for $C_{15}H_{26}O$, to which the authors gave the name hinesol according to the name of original crude drug, "Hineso." Following eluates were composed of mixtures in various ratios of hinesol and eudesmol. The eudesmol fraction crystallised and melted at 79~80°, $[\alpha]_D$ +58.5°. The crystalline sesquiterpenoid alcohol, eudesmol, $C_{15}H_{26}O$, has been found distributed in a number of plants. It shows a rather wide variation in the melting point and specific rotation because even highly purified eudesmol consists of a mixture of α- and β-isomers, apparently in proportions varying with the source. Recently, pure isomers were obtained and clearly characterised.[7] The eudesmol obtained here was very similar to the β-form in its constants, especially in its specific rotation, and showed strong infrared absorptions at 3060 and 833 cm^{-1}, with only small bands at 3025 and 793 cm^{-1}. Therefore, it was considered to consist almost solely of the β-isomer.

The unsaturated sesquiterpenoid alcohol, separated as an oil from the product obtained by the partial reduction of atractylol, must agree to crystalline hinesol but it has never yet crystallised. It may have changed into a liquid during treatment on account of the characteristics to be described below.

Here comes into the question of whether or not either of the components of atractylol underwent any change such as isomerisation during separation through chromatography on alumina and whether the components are a simple mixture or a product which has a tendency to show a constant property (i.e., molecular addition compound).

Then the thermal analysis by thawing-melting method between the two constituents was carried out with the result shown in Fig. 1, in which the melting point of a mixture

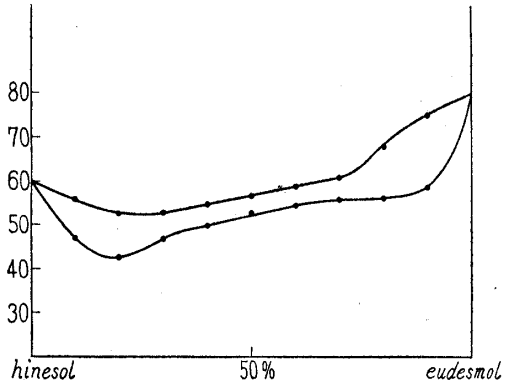

Fig. 1.
Solid-Liquid Equilibrium Diagram
between Hinesol and Eudesmol

was slightly depressed and melting ranges widened because of partial change when the two components were mixed and melted together, on account of unstability of hinesol to heat. For these reasons, the solid-liquid equilibrium diagram was not precise and,

therefore, it is somewhat difficult to conclude definitely from these curves that the formation of a molecular compound between hinesol and eudesmol had taken place, although its formation seems to be quite possible. Further, atractylol may be regarded as not a simple mixture, since the crystals of hinesol are very unstable and rapidly liquefy even at 30° as noted above, while the crystals of atractylol, mixed crystals of eudesmol and hinesol, are comparably stable under the same condition. It is certain at least that the so-called atractylol, m.p. 61~63°, $[\alpha]_D$ +21°±2°, is a difficultly separable mixture of hinesol (ca. 4 parts) and eudesmol (ca. 6 parts). This view was confirmed by the preparation of a mixture of the two alcohols in these proportions, a single crystallisation of which gave an alcohol of m.p. 60~62°, $[\alpha]_D$ +22.0°, identical with the so-called atractylol isolated from a natural source (Fig. 2). The authors there-

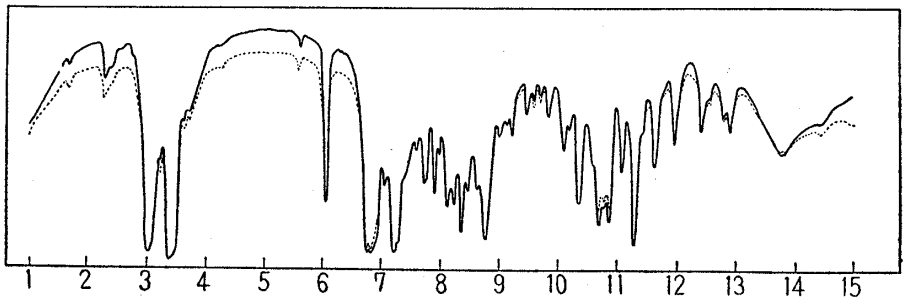

Fig. 2. Infrared Absorption Spectra of Atractylol (in Nujol mull)
—— Natural, from the original oil
------ Regenerated, from hinesol and eudesmol

by believe that hinesol and eudesmol are the very components of atractylol, neither of which underwent change during chromatography.

The authors thank Prof. Dr. L. Ruzicka and Prof. Dr. O. Jeger, Laboratorium für organische Chemie der Eidg. Technishe Hochschule, who very kindly provided the authentic specimens of eudesmol and its dihydro derivative for the identification. Elemental analyses and infrared spectral measurements were carried out by the members of Microanalytical Laboratory and of Photometric Laboratory of this Faculty, to whom thanks are due.

Experimental*3

Catalytic Reduction of Atractylol with Adams Catalyst in Methanol—Atractylol (5.00 g.) in MeOH (25 cc.) was shaken in H_2 and Pt (reduced from 50 mg. of Adams' PtO_2) for 2 hr.; the uptake of H_2 being 279 cc. (theor. for 1 double bond : 503.5 cc., calculated as $C_{15}H_{26}O$(F)). The recovered oil, yet exhibiting a strong yellow color to $C(NO_2)_4$ and immediately discoloring Br_2 solution, gave on distillation a fraction of $b.p_2$ 121~123°, which partially crystallised on being allowed to stand. This material was chilled in diluted acetone solution to −60° with dry ice-acetone mixture and needle crystals (I) were removed from the oily portion (II) by continuous precipitation. Five recrystallisations of the needle crystals (I) from acetone gave colorless needles, m.p. 85~86°, $[\alpha]_D$ +16.8° (c=10.0). *Anal.* Calcd. for $C_{15}H_{28}O$: C, 80.29; H, 12.58. Found : C, 80.21; H, 12.52.

Identity of this substance with the specimen of dihydroeudesmol was established by mixed m.p. and comparison of their infrared spectra.

After the crystalline dihydroeudesmol (I) was removed, the residual oil (II) was distilled under a reduced pressure to afford an oily sesquiterpenoid alcohol, $b.p_4$ 129°, d_4^{25} 0.972, n_D^{25} 1.502, $[\alpha]_D$ −23.6°. *Anal.* Calcd. for $C_{15}H_{26}O$: C, 81.02; H, 11.79. Found : C, 80.99; H, 11.66. This alcohol gave a positive $C(NO_2)_4$ and Br_2 tests.

Catalytic Reduction of Atractylol with Adams Catalyst in Glacial Acetic Acid—Atractylol (5.00 g.) was hydrogenated over Adams' PtO_2 (50 mg.) in AcOH, taking up 1 mole (513 cc.) of H_2. After removal of the catalyst, the product was extracted with ether and evaporation of ether gave a colorless oil, showing a very weak color with $C(NO_2)_4$ and scarcely decolorising Br_2 solution.

Catalytic Reduction of Atractylol with Palladised Charcoal in Methanol—Atractylol (5.00 g.) was hydrogenated in the presence of Pd-C (20%; 0.4 g.) in MeOH (25 cc.), taking up 1 mole (513 cc.) of H_2 in 10 hr. The catalyst and solvent were romoved and the residue on distillation yielded

*3 All m.p.s and b.p.s are uncorrected. Optical rotations were measured in $CHCl_3$.

a colorless oil, which was almost saturated to $C(NO_2)_4$ and Br_2.

Chromatography of Atractylol; Separation of Atractylol into Two Components—Atractylol, m.p. 61~63° (5.0 g.), was dissolved in light petroleum (b.p. 40~70°) and adsorbed on a column of active alumina (120 g.). Elution of the column with light petroleum liberated no material. Light petroleum-benzene yielded (a) stout needle crystals (1.1 g.), which, after repeated crystallisation from hydr. MeOH, formed white needles, m.p. 59~60°, $[\alpha]_D$ −40.2(c=10.0). *Anal.* Calcd. for $C_{15}H_{16}O$: C, 81.02; H, 11.79. Found : C, 80.84, 80.77; H, 11.53, 11.65.

Elution with benzene gave (b) white mass of crystals (2.3 g.), which was a mixture of (a) and (c). Elution with EtOH gave (c) as colorless vitreous substance (1.6 g), which slowly crystallised into a solid mass, m.p. 60~72°. Several crystallisations of (c) from hydr. MeOH gave white needles, m.p. 79~80°, unchanged by further crystallisation, $[\alpha]_D$ +58.5°(c=10.0). *Anal.* Calcd. for $C_{15}H_{26}O$: C, 81.02; H, 11.79. Found : C, 80.80; H, 11.83.

This was identified with eudesmol by m.p., mixed m.p., specific rotation, and infrared absorption.

Hinesol-Eudesmol Mixture; Regeneration of Atractylol—A mixture of hinesol (104 mg., 0.38 mole) and eudesmol (186 mg., 0.62 mole), melted together, showed $[\alpha]_D$ +20.2°(c=12.9), and recrystallised once from hydr. MeOH to white needles, m.p. 60~62°, $[\alpha]_D$ +22.0°(c=10.0). The mixed crystal obtained by this method was identical in mixed m.p. test and comparison of infrared spectra with atractylol derived from the original oil.

Summary

The sesquiterpenoid alcohol, the so-called atractylol, a major constituent of the essential oil from the rhizomes of *Atractylodes lancea* De Candolle (Compositae), was separated into the known eudesmol and a new sesquiterpenoid alcohol, hinesol, which were characterised. Association of the two alcohols was established unambiguously by the regeneration of the so-called atractylol from them.

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62. Sadao Iguchi, Kazuhito Hisatsune, Michiko Himeno, and Shigeteru Muraoka : Studies on Pyrone Derivatives. III.*1 On the Reaction of Dehydroacetic Acid to Amino Acids. (1).

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As was briefly reported in a previous communication,[1] it is thought interesting, in considering the powerful antibacterial and antifungal properties, that both dehydroacetic acid (DHA) and 3-acetyl-4-hydroxycoumarin (AHC), analogical in their structure, react with ammonia, amines, various amino acids, and with some aromatic amino compounds forming reaction products under a mild condition. In this paper, details of such a reaction between DHA or AHC and amino compound are described and the structure of reaction products is also discussed.

It was already reported by Oppenheim, *et al.*[2~5] that DHA reacted with conc. ammonia, producing 3-(1-iminoethyl)-4-hydroxy-6-methyl-2-pyrone(DHA-imide)(II), but not with dil. ammonia. However, it was found that this reaction also proceeded under a milder condition, i.e. in dil. aqueous ammonia (below 5%) at room temperature and the

*1 Part II : Yakugaku Zasshi, **77**, 98(1957).
*2 Katakasu, Fukuoka (井口定男, 久恒和仁, 姫野道子, 村岡重輝).
1) S. Iguchi, K. Hisatsune : Yakugaku Zasshi, **77**, 1258(1957).
2) Oppenheim, Precht : Ber., **9**, 1100(1876).
3) Collie, Myers : J. Chem. Soc., **63**, 128(1893).
4) Feist : Ann., **257**, 253~297(1890).
5) Petrenko-Kritschenko, Schöttle : Ber., **44**, 2830(1911); *ibid.*, **45**, 3231(1912).