Notes

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During the course of an investigation on anthraquinone derivatives present in the fresh rhizome of rhubarb (*Rheum coreanum* NAKAI), separation of anthraquinones was effected by the difference in their solubility in alkaline solutions and it was unexpectedly observed that aloe-emodin, 1,8-dihydroxy-3-hydroxymethylanthraquinone, was completely extractable with sodium carbonate solution.

It has been known that anthraquinone derivatives having a hydroxyl group in the β-position are soluble in aqueous alkaline carbonate, while anthraquinones possessing a hydroxyl only in the α-position are insoluble in the carbonate solution but soluble in alkali hydroxide solution. It is also known that a β-hydroxyl group is methylated with diazomethane, whereas an α-hydroxyl is hardly affected by the reagent, being chelated with the quinonecarbonyl function. Such difference of properties dependent on the position of hydroxyl group has proved its applicability in isolation of anthraquinone derivatives from natural materials and clarification of their chemical structures.

According to this empirical knowledge, a compound (I) of m.p. 218∼219°, $C_{15}H_{10}O_5$, obtained in the present series of experiment from the fraction soluble in sodium carbonate solution, was presumed to be not aloe-emodin but a β-hydroxylated anthraquinone. Methylation of (I) with diazomethane afforded a monomethyl ether, m.p. 217∼218°, which was characterized by elemental analysis and infrared spectrum. Treatment of the monomethyl ether with dimethyl sulfate and sodium carbonate[*2] resulted in methylation of the α-hydroxyls, giving a dimethyl ether, m.p. 221∼223.5°, and further methylation under a more drastic conditions using dimethyl sulfate and sodium hydroxide[*3] led to the formation of a crystalline trimethyl ether, m.p. 153∼156°. The melting point and analytical data of the product were found to be in agreement with those of aloe-emodin trimethyl ether. Acetylation of (I) with acetic anhydride and pyridine furnished a triacetate, m.p. 174∼177°, which agreed with the melting point reported for aloe-emodin triacetate. Finally, oxidation with chromium trioxide converted the triacetate of (I) into rhein diacetate, identification being made by direct comparison with an authentic sample.

The above results proved the identity of the original compound (I) with aloe-emodin. However, the fact that the compound (I), both as pure crystals and as a solution in organic solvents, is completely extractable with alkali carbonate solution and forms an α-methoxyl derivative[*4] by treatment with diazomethane, is inconsistent with the properties recognized so far for anthraquinone derivatives. It now became clear, therefore, that separation with alkaline solution and methylation with diazomethane should be utilized only as diagnostic means in the structural study of unknown anthraquinone compounds

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[*2] The conditions for methylation of an α-hydroxyl group.

[*3] Methylation of the hydroxyl in hydroxymethylanthraquinones requires in general such drastic conditions. cf. T. Posternak: Helv. Chim. Acta, **23**, 237, 1046 (1940). Benzyl alcohol can be converted to benzyl methyl ether under similar conditions. cf. R. Pschorr, F. Dickhäuser: Chem. Ber., **44**, 2633 (1911).

[*4] That the methylation took place at the α-hydroxyl group is evident from the fact that the monomethyl ether exhibits a hydroxyl band in the infrared spectrum and is soluble in sodium carbonate solution.

and care must be taken when a conclusion is drawn only from experimental data obtained by those means.

In 1899, Hesse[1] isolated from rhubarb a compound, m.p. 212°, $C_{15}H_{10}O_5$, soluble in sodium carbonate solution and named it rhabarberone. A product of similar properties, isoemodin, was obtained by Eijken[2] in 1904 and he supposed it to be identical with rhabarberone.[3] Following a detailed examination of rhubarb, Tutin and Clewer[4] suggested in 1911 that rhabarberone and isoemodin were merely impure samples of aloe-emodin. Hesse,[5] however, regarded aloe-emodin and rhabarberone as distinctly different substances, the latter being soluble in sodium carbonate solution. The present experiment revealed that aloe-emodin is soluble in carbonate solution and thus the identity of rhabarberone and isoemodin with aloe-emodin has become all the more probable. Re-investigation of these old preparations is impossible, but the fact that occurrence of those compounds has never been reported*5 since then may be evidence that the compounds are aloe-emodin.

In this connection, Keimatsu and Hirano[6] synthesized 2-methyl-3,5,8-trihydroxyanthraquinone and claimed it to be rhabarberone and isoemodin as it had the same melting point, but it was not compared directly with the natural materials. This structure seems improbable from the present data as well as on phytochemical or biogenetic grounds[7] as Thomson indicated,[8] and the coincidence in the melting points is probably accidental.

Experimental*6

Aloe-emodin—The EtOH extract of the fresh rhizome of ***Rheum coreanum*** Nakai was extracted with $CHCl_3$ and the anthraquinone mixture obtained was shaken with 10% $NaHCO_2$ solution and then with 10% Na_2CO_3 solution. The Na_2CO_3 extract was acidified and extracted with $CHCl_3$. The residue obtained upon evaporation of $CHCl_3$ was subjected to fractional recrystallization from Me_2CO-MeOH mixture. Aloe-emodin was obtained as dark orange granules, m.p. 218~219°. UV λ_{max}^{EtOH} mμ (log ε): 226(4.60), 256(4.33), 288(4.05), 430(4.07); IR λ_{max}^{KBr} μ: 2.95(OH), 5.95(non-conjugated CO), 6.13 (conjugated CO). *Anal.* Calcd. for $C_{15}H_{10}O_5$: C, 66.67; H, 3.73; O, 29.60. Found: C, 66.66; H, 3.86; O, 29.78. From the mother liquor, emodin, m.p. 257~259°, was obtatained.

The $CHCl_3$ layer from the above Na_2CO_3 extraction did not contain aloe-emodin any more as proved by paper chromatography and examination of NaOH-soluble substances present in the $CHCl_3$ solution.

Aloe-emodin Monomethyl Ether—A solution of 100 mg. of aloe-emodin in 70 cc. of Me_2CO was mixed with the Et_2O solution of CH_2N_2 (prepared from 300 mg. of nitrosomethylurea and 10 cc. of Et_2O) and the mixture was allowed to stand at room temperature for 2 days. The reaction mixture was treated in usual manner and, after one recrystallization from MeOH, 40 mg. of yellow granules, m.p. 180~205°, was obtained which was purified by 3 more recrystallizations to material of analytical quality, m.p. 217~218°. IR λ_{max}^{KBr} μ: 2.75(OH), 5.95(non-conj. CO), 6.10(conj. CO). *Anal.* Calcd. for $C_{16}H_{12}O_5$: OCH_3, 10.90. Found: OCH_3, 10.89.

Aloe-emodin Dimethyl Ether—To 30 mg. of the monomethyl ether, 30 cc. of Me_2CO, 2 g. of anhyd. K_2CO_3, and 0.5 cc. of Me_2SO_4 were added and the mixture was heated for 4 hr. with stirring. The mixture, after addition of 1 g. of K_2CO_3 and 0.5 cc. of Me_2SO_4, was refiuxed for another 3 hr. and

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*5 From cascara sagrada and Curaçao aloe there has been obtained a compound named isoemodin but it seems to have no relationship to the substances in the present consideration. cf. M. W. Green, C. G. King, G. D. Beal: J. Am. Pharm. Assoc., Sci. Ed., **27**, 95 (1938); M. R. Gibson, A. E. Schwarting: *Ibid.*, **37**, 206 (1948); T. M. Brody, R. F. Voigt, F. T. Maher: *Ibid.*, **39**, 666 (1950).

*6 All m.p.s are uncorrected.

1) O. Hesse: Ann., **309**, 32 (1899).
2) P. A. A. F. Eijken: Pharm. Weekblad, **41**, 177, 197 (1904).
3) A. Tschirch, P. A. A. F. Eijken: Schweiz. Wochshr. Pharm., **1904**, Nos. 40 and 41.
4) F. Tutin, H. W. B. Clewer: J. Chem. Soc., **99**, 946 (1911).
5) O. Hesse: J. prakt. Chem., **77**, 383 (1908).
6) S. Keimatsu, I. Hirano: Yakugaku Zasshi, **49**, 147 (1929); **51**, 230 (1931).
7) A. J. Birch: Fortschr. Chem. org. Naturstoffe, **14**, 186 (1957).
8) R. H. Thomson: "Naturally Occurring Quinones," 177 (1957). Butterworths Scientific Publications, London.

left to stand overnight. After treatment in conventional way, the reaction product was recrystallized from MeOH to yellow needles, m.p. 221~223.5°. IR λ_{max}^{Nujol} μ: 2.86(OH), 5.99(non-conj. CO). *Anal.* Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73; $(OCH_3)_2$, 20.81. Found: C, 68.10; H, 4.97; OCH_3, 21.29.

Aloe-emodin Trimethyl Ether—A mixture of 130 mg. of aloe-emodin, 7.5 cc. of *N* NaOH solution, and 1 cc. of Me_2SO_4 was stirred at room temperature for 1.5 hr. One-third portion each of a solution of 1.5 cc. of Me_2SO_4 in 7.5 cc. of *N* NaOH was added three times to the reaction mixture in an interval of 2 hr., during which stirring was continued. After being left overnight, the mixture was again stirred with another 0.5 cc. of Me_2SO_4 and 5 cc. of 2*N* NaOH solution for 8 hr. and finally mixed with 0.5 cc. of Me_2SO_4 and 3 cc. of 2*N* NaOH solution, and left to stand overnight. Treatment of the reaction mixture in a usual way and recrystallization of the product from MeOH afforded orange needles, m.p. 153~156°. IR λ_{max}^{Nujol} μ: 5.98(non-conj. CO). *Anal.* Calcd. for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16; OCH_3, 29.77. Found: C, 69.39; H, 5.29; OCH_3, 29.86.

Aloe-emodin Triacetate—Acetylation of 70 mg. of aloe-emodin was performed with 2.75 cc. each of Ac_2O and pyridine in a conventional manner and after recrystallization from Me_2CO-MeOH mixture, 50 mg. of the triacetate was obtained as pale yellow needles, m.p. 174~177°. IR λ_{max}^{Nujol} μ: 5.64, 5.75, 5.93, 5.97(acetate and CO). *Anal.* Calcd. for $C_{21}H_{16}O_5$: C, 63.63; H, 4.07. Found: C. 63.91; H, 4.27.

Rhein Diacetate from Aloe-emodin Triacetate—To a solution of 553 mg. of aloe-emodin triacetate in 15 cc. each of AcOH and Ac_2O, a solution of 550 mg. of CrO_3 in 15 cc. of AcOH and 1 cc. of H_2O was added dropwise at 55° in the course of 30 min. The mixture was stirred at 65~70° for 3 hr. and poured into lukewarm water. The precipitate was collected and recrystallized from dioxane to 320 mg. of yellow granules, m.p. 247~247.5°(decomp.). IR λ_{max}^{Nujol} μ: 5.65(acetate), 5.88(COOH), 5.93 (CO). *Anal.* Calcd. for $C_{19}H_{12}O_8$: C, 61.96; H, 3.29. Found: C, 62.17; H, 3.49. The product was confirmed to be identical with authentic rhein diacetate, m.p. 250°(decomp.), by mixed m.p. determination and infrared spectrum.

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Summary

An unexpected finding is reported that aloe-emodin is soluble in aqueons sodium carbonate solution and is methylated with diazomethane. From these results, identity of rhabarberone and isoemodin with aloe-emodin is suggested.

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Takao Inoue and Yaeko Kawamura: Studies on Biogenesis of Tea Components. II.⁽¹⁾ Formation of Caffeine in Excised Tea Shoots.

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Biosynthesis of plant alkaloids has been studied by numerous workers. Dawson⁽²⁾ early demonstrated that nicotine formed in the roots of Nicotiana but not in the leaves, while similar results were obtained with Datura alkaloid. Imaseki,⁽³⁾ however, recently confirmed that Datura alkaloids are partly biosynthesized in the leaves in addition to the roots, using a tracer technique.

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1) Part I: Yakugaku Zasshi, **80**, 548 (1960).

2) R.F. Dawson: Am. J. Bot., **31**, 351 (1944); S.M. Peacock, Jr., D.B. Leyerle, R.F. Dawson: *Ibid.*, **31**, 463 (1944).

3) I. Imaseki: This Bulletin, **5**, 447 (1957).