pillars, m.p. 100.5~101°. *Anal.* Calcd. for $C_{14}H_{18}ON_4$: C, 65.09; H, 7.02; N, 21.69. Found : C, 65.55; H, 7.26; N, 21.68. IR ν_{max}^{Nujol} cm^{-1} : 3360, 3320, 3230 (NH_2), 1650 (broad) (Amide I, NH_2), 1523 (Amide II), 990, 910 (CH_2=CH-), 730 (*o*-disubstituted benzene).

1-Benzyltryptophan Hydrazide Sulfate (IVd)——The hydrazide was synthesized from 1-benzyltryptophan methyl ester and anhyd. hydrazine in EtOH, but failed to crystallize. IR $\nu_{max}^{Cap.}$ cm^{-1} : 3345 (broad) (NH_2), 1670 (broad) (Amide I), 1613 (NH_2), 1555 (Amide II), 740 (broad), 699 (benzene).

Sulfate was obtained from equivalent molecules of the crude hydrazide and 95% aq. H_2SO_4 in EtOH-Et_2O (1:2). Yield was quantitative. Recrystallizations from 30% aq. EtOH (charcoal) afforded colorless granules, showing m.p. 222° (decomp.). *Anal.* calcd. for $C_{18}H_{20}ON_4 \cdot H_2SO_4$: C,53.19; H, 5.46; N, 13.79. Found: C, 53.24; H, 5.53; N, 13.50. IR ν_{max}^{Nujol} cm^{-1} : 3200~2600 (broad) (NH_3^+), 1708 (Amide I), 1609 (NH_3^+), 1549 (Amide II), 1111, 1064, 1030 (SO_4^{--}?), 732, 700 (benzene).

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Summary

A convenient method for the synthesis of 1-alkyltryptophans is described. The proof of ind.-N-alkylation was obtained from several data. Effect of 1-alkyl substitution of indole was observed on the ultraviolet spectra of 1-alkyltryptophans. It was confirmed by debenzylation of L-1-benzyltryptophan in liquid ammonia that no racemization had occurred in the alkylation. 1-Alkyltryptophans were converted to hydrazides *via* methyl ester hydrochlorides in good yields.

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Yutaka Fujise, Takashi Toda, and Shô Itô : Isolation of Trifolirhizin from *Ononis spinosa* L.

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In an attempt to isolate α-onocerin from material identified as *Ononis spinosa* L., Professor Stork at Columbia University obtained an acetate, m.p. 188~189°, which on alkaline hydrolysis afforded a compound, m.p. 223°, different from α-onocerine, m.p. 202~203°. Described in this paper is a chemical investigation of these compounds, generously provided by Professor Stork, which has shown them to be identical with trifolirhizin (maackiain glucoside) and its tetraacetate.

We have encountered some difficulty in obtaining constant figures for the elementary analyses of the compound of m.p. 223° (I) because of its great tendency to occlude solvent molecules. The melting point of the compound also varied considerably depending on the state of dryness. Finally, after for a long time at an elevated temperature, constant analytical values were obtained to establish the molecular formula as $C_{22}H_{22}O_{10}$. On the other hand, micro analysis of the acetate (II) presented no difficulty and gave constant figures for a molecular formula of $C_{30}H_{30}O_{14}$ when the molecular weight as determined by osmotic method and the Rast method is taken into consideration. An acetoxyl determination showed the presence of four acetoxyl groups. The infrared spectrum of compound (I) had hydroxyl absorption but none dues to carbonyl groupings, whereas that of the acetate

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(II) exhibited strong bands at 1760 cm^{-1} and 1235 cm^{-1}, due to O-acetate groupings, but no hydroxyl bands. The presence of an aromatic ring in I was implied by (i) the low hydrogen content, (ii) the strong absorption maxima at 284.5 mμ (ε 4390) and 310.5 mμ (ε 7440) in its ultraviolet spectrum, and (iii) bands at 1630, 1580, and 845 cm^{-1} in the infrared region. The ultraviolet maxima were essentially unaffected by acetylation. The aromatic ring is not phenolic since (i) the compound I gives a negative ferric chloride test and (ii) the ultraviolet absorption maxima do not show any shift in acidic or alkaline medium. Compound (I) did not give any of the color reactions characteristic of flavanone. The presence of a methylenedioxy group was established by the positive Rabat test and Hansen test, by its infrared spectrum (bands at 1040, and 934 cm^{-1}) and by the presence of two sets of doublet at 5.89 and 5.90 p.p.m. which coupled each other with J=1.2 c.p.s.

The acetate (II) was converted in good yield to I by alkali or lithium aluminum hydride, but in only 20% yield by acid, whereas treatment of I with acetic anhydride in pyridine afforded a quantitative yield of II. Although I was either unaffected or gave no definite product when subjected to methylation, dehydration, or oxidation by permanganate or chromium trioxide, it afforded styphnic acid when oxidized by nitric acid thus disclosing the presence of two oxygen functions situated in 1,3-position in one of the rings.

The presence of a sugar moiety, implied by (i) the large number of oxygens which must be present as hydroxyl or ether groupings, and (ii) the formation of formaldehyde by periodate oxidation of I, was substanciated by the identification of glucose (by thin-layer chromatography and the formation of a phenylosazone, m.p. 208°) among the products from the acid treatment of I.

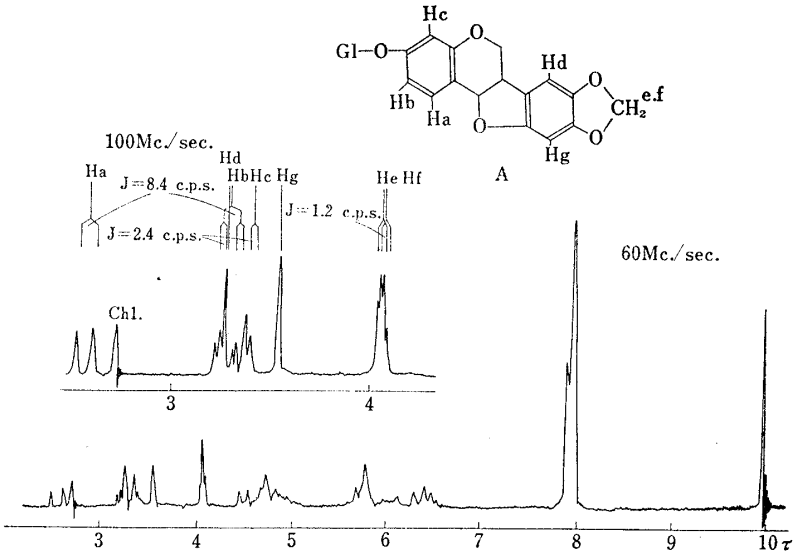

Fig. 1. NMR Spectra of Trifolirhizin in $CDCl_3$

As shown in Fig. 1, the 60 Mc. and 100 Mc. spectra of the acetate II in deutero-chloroform discloses the pattern of substitution in the two aromatic rings. Analysis was straightforward for these spectra which were very similar to those of pterocarpin[1] and homopterocarpin;*2 from this and the evidence given above, structure (A) was deduced. This structure has been previously assigned to trifolirhizin,[2] isolated by Bredenberg, *et al.* from *Trifolium pratense* L., and direct comparison of these two compounds (IR) and their acetates (IR and mixed m.p.) established their identity.

*2 The authentic sample was provided by Drs. H. Suginome and M. Takasugi, Hokkaido University, to whom author's thanks are due.

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Experimental

Trifolirhizin Tetraacetate (II)——Isolation from the plant material was carried out by G. Stork and his collaborators following the procedure used by Barton, *et al.*[3] in the isolation of α-onocerin. Recrystallized from MeOH-CCl_4. Colorless needles, m.p. 189°, $[\alpha]_D^{20}$ −137° (c=5, dioxane). *Anal.* Calcd. for $C_{30}H_{30}O_{14}$: C, 58.63; H, 4.92; mol. wt., 614.54. Found: C, 58.67; H, 4.64; mol. wt., 617.7 (osmotic method), 604, 634 (Rast method). UV λ_{max}^{MeOH} mμ (log ε): 310.5 (3.84), 284.5 (3.58). IR ν_{max}^{KBr} cm^{-1}: 1760, 1235 ($OCOCH_3$).

Trifolirhizin (I)——This was prepared from the acetate (II) by the following methods.

a) $LiAlH_4$ reduction: The acetate (II) (500 mg.) and $LiAlH_4$ (500 mg.) in tetrahydrofuran (50 ml.) was stirred at room temperature for 1.5 hr., and then treated with an excess of AcOEt. After dilution with H_2O, the organic layer was washed successively with dil. H_2SO_4, satd. $NaHCO_3$, and H_2O, and then dried. The residue obtained on evaporation crystallized when treated with acetone. Recrystallization from MeOH gave trifolirhizin as colorless needles (285 mg. 78% yield), m.p. 223° after being dried at 150°/2 mm. Hg for 20 hr. $[\alpha]_D^{17.3}$ −181° (c=2, acetone). *Anal.* Calcd. for $C_{22}H_{22}O_{10}$: C, 59.19; H, 4.97; mol. wt., 446.40. Found: C, 59.46; H, 4.92; mol. wt., 476 (Rast method). UV λ_{max}^{MeOH} mμ (log ε): 310.5 (3.87), 284.5 (3.64). IR ν_{max}^{KBr} cm^{-1}: 3340 (OH), 1630, 1580, 845 (aromatics), 1040, 934 (-O-CH_2-O-).

b) Alkaline hydrolysis: Acetate (100 mg.) was heated under reflux with 6*N* NaOH (1 ml.) in MeOH (50 ml.) for 2 hr. After concentration of the solvent, and dilution with H_2O, the reaction mixture was extracted with AcOEt. Treatment of the extract as described in the previous section yielded I (96 mg.) in 70% yield.

c) Acid hydrolysis: Acetate (50 mg.) was heated under reflux with 2*N* H_2SO_4 (2 ml.) in MeOH (40 ml.) for 4 hr., and then treated as described in b) to afford 8 mg. (22%) of the compound I.

Periodate Oxidation of I——A solution of I (10 mg.) in 1 ml. of 0.18 mol. solution of periodic acid in 75% of dioxane was diluted with 3 ml. of H_2O, and then distilled with steam. The distillate was treated with 2,4-dinitrophenylhydrazine hydrochloride, and the hydrazone which precipitated was purified by alumina chromatography to give formaldehyde 2,4-dinitrophenylhydrazone, m.p. 157~159°.

Nitric Acid Oxidation of I——Trifolirhizin (100 mg.) was heated on the water bath for 4 hr. with 3 ml. of nitric acid. The resulting yellow solution was diluted and then evaporated to dryness. The yellow crystalline residue (20 mg.) was recrystallized from EtOH to give pale yellow plates, m.p. 176°. *Anal.* Calcd. for $C_6H_3O_8N_3$: C, 29.40; H, 1.23; N, 17.14. Found: C, 29.68; H, 1.47; N, 16.92. A comparison of their UV and IR specrta and pK values, and a mixed melting point test showed this compound to be identical with authentic styphnic acid.

Acid Treatment of I——A mixture of an AcOH solution (100 ml.) of I (500 mg.) and conc. HCl (10 ml.) was left at room temperature for 20 hr. and then heated on a water bath for 30 min. After being allowed to cool, the reaction mixture was diluted with H_2O and extracted with ether. Aqueous layer was neutralized with aq. NaOH and then evaporated. The residue was digested with abs. EtOH and decolorized with active charcoal. Evaporation of the solvent gave a brown oil. Thin-layer chromatography on silica gel (developer: AcOEt-iso-PrOH-H_2O=1:2:1. Reagent: anisaldehyde-sulfuric acid) afforded a spot at Rf 0.62 coincident with that of glucose. The oil afforded an phenylosazone, m.p. 208°, after two recrystallizations from EtOH. The osazone was identified as glucosazone by mixed melting point test and comparison of their IR spectra.

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