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# ILLUDIN S (LAMPTEROL)[1]

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**Abstract**—An antitumour factor, lampterol, $C_{15}H_{20}O_4$, has been isolated from the poisonous mushroom, *Lampteromyces japonicus* (KAWAM.) Sing. During the structural studies on lampterol, structures were proposed for illudin S, which was found to be identical with lampterol. The same conclusion was reached by independent chemical and spectroscopic data, and recently by X-ray analysis. The unique structure of illudin S (lampterol) gives rise to several rearrangement products. Analysis of optical and spectroscopic data enables one to deduce the full stereochemistry of illudin S as represented by I.

TSUKI-YO-TAKE (moon-night-mushroom, *Lampteromyces japonicus* (KAWAM.) Sing., is a bioluminescent mushroom that grows on rotten beech trees during the month of October. It is toxic and has been the cause of fatal accidents because of its similarity to common edible mushrooms.

Isolation of the toxic principle in a semi-pure state was reported by Nakai in 1958[3] and the present investigation was undertaken in order to characterize this toxin. During an intensive screening for antitumour substances of about 600 mushrooms, *L. japonicus* was shown to possess high activity,[4] and accordingly extractions for both the toxic and antitumour principles were carried out independently. At the final stages of fractionation it became clear that the two substances are identical. Isolation of the active principle, lampterol,[5] is best carried out by soaking the mushroom in methanol immediately after collection[6] and then processing as described in the Experimental. Thus 300 kg of the mushroom finally give 6·2 g of lampterol, m.p. 124–126°. The activity is 120 $\gamma$/kg as measured by the effect on Ehrlich mouse ascitic tumours, and the toxicity 5 mg/kg on intraperitoneal injection into ordinary mice. Matsumoto *et al.* also isolated the same substance from the same source[7] and named it lunamycin but it was agreed to call it lampterol. During our structural studies on lampterol, structures were proposed[8] for illudin S and M which had been isolated in 1950 from

[1] The contents have been partly published as preliminary communications.[11,12]

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[3] K. Nakai, *Medicine and Biology* **48**, 231 (1958); **49**, 129 (1959) in Japanese.

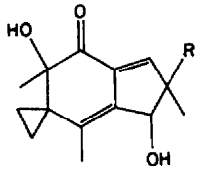
[4] N. Komatsu, M. Hamada, S. Nakasawa, S. Ogata, A. Yamamoto, H. Terakawa and T. Yamamoto, *J. Ferment. Assoc.* **19**, 464 (1961) in Japanese.

[5] K, Nakanishi, M. Tada, Y. Yamada, M. Ohashi, N. Komatsu and H. Terakawa, *Nature, Lond.* **197**, 292 (1963).

[6] K. Nakanishi, M. Ohashi, N. Suzuki, M. Tada, Y. Yamada and S. Inagaki, *Yakugaku Zasshi* **83**, 377 (1963).

[7] S. Shirahama, Y. Fukuoka and T. Matsumoto, *Bull. Chem. Soc., Japan* **35**, 1047 (1962); *Nippon Kagaku Zasshi* **83**, 1289 (1962).

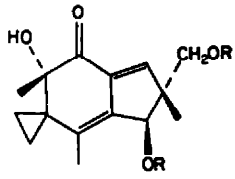
[8] T. C. McMorris and M. Anchel, *J. Amer. Chem. Soc.* **85**, 831 (1963).




Illudin S, R = $CH_2OH$
Illudin M, R = $CH_3$

*Clitocybe illudens* by Anchel *et al.* The same conclusion was reached by independent investigations, and a subsequent direct comparison[9] showed lampterol and illudin S to be identical. The unique structure has received full support from an X-ray analysis[10] on the *p*-iodobenzoate of its acyloin rearrangement product, isoilludin S (isolampterol). Although the X-ray crystallographic results to date do not clarify the full stereochemistry, i.e., relative and absolute configurations, this becomes possible when various optical data are considered as well. The present paper describes corroborative evidence for the structure[11], some reactions,[11] and the full stereochemistry[12,13] of illudin S (lampterol).

Illudin S (I), m.p. 124–126°, $C_{15}H_{20}O_4$, $M^+$ peak at 264, $\lambda_{max}^{MeOH}$ 235, 320 m$\mu$ (log $\varepsilon$ 4·10, 3·54) forms a diacetate II upon treatment with acetic anhydride and pyridine. The nature of all 20 protons of illudin S are easily disclosed by comparing the NMR spectrum with that of its diacetate.




I R = H
II R = $COCH_3$

The NMR spectra of illudin S and its diacetate show a resonance at 2·37 ppm corresponding to 3 protons and at 3·52 ppm representing one proton, respectively. These signals are assigned to hydroxyl protons since these peaks can easily be removed when the samples are shaken with a little $D_2O$. The single absorption at 3·48 representing two protons in the illudin S spectrum becomes an AB type quartet[14] at 3·99 ppm in the diacetate spectrum. Furthermore, the signal at 4·70 ppm corresponding to one proton in the spectrum of the parent compound shifts to 5·80 ppm in the acetate trace.

[9] Private communication from Professor T. Matsumoto, Hokkaido University.

[10] Private communication from Professor Y. Saito, University of Tokyo.

[11] M. Tada, Y. Yamada, M. Ohashi, N. S. Bhacca and K. Nakanishi, *Chem. Pharm. Bull.* **12**, 853 (1964).

[12] K. Nakanishi, M. Tada and Y. Yamada, *Chem. Pharm. Bull.* **12**, 856 (1964).

[13] The same *relative* configurations for the 3 optical centres have been deduced by Matsumoto and co-workers: Y. Fukuoka, A. Ichihara, T. Matsumoto, Y. Mori, H. Shirahama, Y. Takahashi, M. Watanabe, IUPAC Symposium on the Chemistry of Natural Products, Kyoto, 1964, Abstracts p. 23.

[14] This phenomenon, i.e., the conversion of a two-proton singlet of a hydroxymethyl group to an AB quartet upon acetylation, is characteristic of a $CH_2OR$ attached to an asymmetric centre. Theoretically the C*—$CH_2OH$ protons should also constitute an AB quartet; however, the rapid rotation around the C*—C bond renders the chemical shifts of the two protons very similar and this makes the two centre lines of the quartet to approach each other (the two satellites conversely are greatly reduced in intensity).

TABLE 1. NMR SPECTRA OF ILLUDIN S (I) AND DERIVATIVES, $CDCl_3$, TMS INTERNAL REFERENCE, 60 mc

| | | illudin S | illudin S diacetate | isoilludin S | isoilludin S triacetate |
|---|---|---|---|---|---|
| $H_2$ $H_2$ | 4 H | 0·3–1·4 (m) | 0·3–1·4 (m) | 1·2 (m) | 1·3 (m) |
| $+CH_3$ | 3 H | 1·19 (s) | 1·12 (s) | 1·17 (s) | 0·99 (s) |
| OH $CH_3$ | 3 H | 1·37 (s) | 1·37 (s) | 1·49 (s) | 1·49 (s) |
| $=CH_3$ | 3 H | 1·68 (s) | 1·51 (s) | 1·67 (s) | 1·52 (s) |
| $-OCOCH_3$ | | | 1·97 [3 H]<br>2·05 [3 H] | | 2·01 [6 H]<br>2·09 [3 H] |
| $-OH$ | | 2·37 [3 H] | 3·52 [1 H] | 2·22 [3 H] | |
| $+CH_2OH$ | 2 H | 3·48 (s) | A: 3·85<br>B: 4·13<br>(J: 11·4) | 3·40 (s) | A: 3·82<br>B: 4·13<br>(J: 11·4) |
| OH H | 1 H | 4·70 (s) | 5·80 (s) | 4·60 (s) | 5·42 (s) |
| $=H$ | 1 H | 6·44 (s) | 6·43 (s) | 5·69 (s) | 5·90 (s) |

m: multiplet; s: singlet; d: doublet, integrated intensity in [ ].

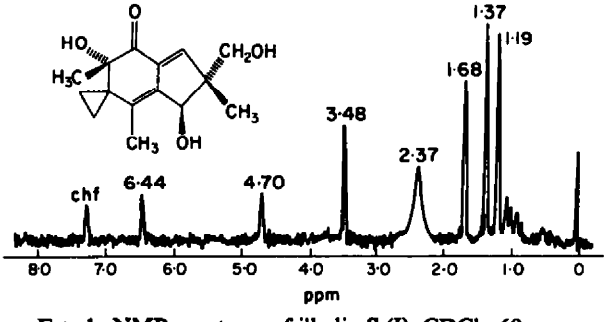
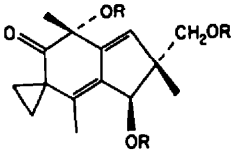



FIG. 1. NMR spectrum of illudin S (I), $CDCl_3$, 60 mc.

The comparison of the chemical shifts of the above resonances in the two spectra indicate that illudin S contains a quarternary hydroxymethyl, an allylic secondary hydroxyl and a tertiary hydroxyl group. The single peak around 6·4 ppm in the two spectra may be assigned to an olefinic proton in the $\beta$-position of an $\alpha\beta$-unsaturated ketone, while the rest of the tall signals at 1·19, 1·37 and 1·68 ppm in illudin S and 1·12, 1·37, 1·51, 1·97 and 2·05 ppm in the diacetate spectrum correspond to various methyl groups as indicated in Table 1. The small multiplets around 0·4 ppm and 1·0 ppm representing 1 and 3 protons, respectively, are assignable to 4 cyclopropane

ring protons. Various derivatives of illudin S and isoilludin S show IR bands around 3050 $cm^{-1}$, confirming the presence of the cyclopropane methylene group; presence of the spirocyclopropane moiety is established by the production of cyclopropane-1,1-dicarboxylic acid upon oxidation of illudin S with potassium permanganate.

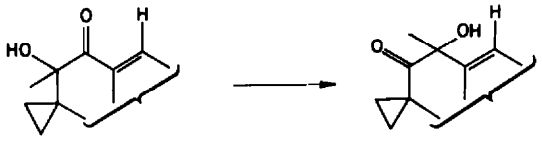
When illudin S is heated on a hot stage, or when its chloroform solution is passed through an alumina column, it gives the isomer isoilludin S (III), m.p. 179–180°. This isomer, in contrast to illudin S, forms a triacetate (IV), upon acetylation with acetic anhydride and pyridine. The NMR spectra of illudin S and isoilludin S (or their




III R = H
IV R = Ac

acetates) are similar except that in the isomer the cyclopropyl protons are shifted lower by 0·7 ppm whereas the olefinic proton is shifted higher by 0·5 ppm (Table 1). It is apparent from the NMR data that the tertiary hydroxyl is also acetylated in the isomer, and this difference towards acetylation suggests that the isomerization involves a change in the moiety surrounding the tertiary hydroxyl group. The fact that this tertiary hydroxyl is contained in an α-ketol group is suggested by the consumption of one mole equivalent periodic acid by illudin S diacetate (II), as well as by illudin S (I) and isoilludin S (III) to afford carboxylic acids (with positive iodoform test). The IR spectra of illudin S and its diacetate in $CCl_4$ solution show concentration-independent bands at 3510 and 3508 $cm^{-1}$, respectively, and this observation is in accord with the presence of an intramolecular hydrogen-bonding.

The IR spectrum of isoilludin S (III), $\nu^{KBr}$ 3400 (broad), 1697 (C=O) and 1645 $cm^{-1}$ (C=C), significantly lacks the strong band at 1606 $cm^{-1}$ present in the spectrum of illudin S (I) and which is characteristic for the double bond of a cisoid α,β-unsaturated ketone. In addition, the UV maximum of the isomer appears at 252 mμ (log ε 4·13, in EtOH), and is not changed when measured in ethanolic $NaBH_4$.[15] The




illudin S isoilludin S

evidence from NMR, IR, UV and periodic acid oxidation suggests that the following acyloin rearrangement of the α-ketol group, involving migration of the tertiary methyl, has taken place during isomerization. Similar examples of acyloin rearrangements upon treatment with alumina[16a,16b] or heat[17] have been reported in the literature.

[15] O. R. Vail and D. M. S. Wheeler, *J. Org. Chem.* 27, 3803 (1962).
[16a] N. L. Wendler, *Tetrahedron* 11, 163 (1960).
[16b] R. B. Turner, *J. Amer. Chem. Soc.* 75, 3484 (1953).
[17] N. L. Wendler, *Chem. & Ind.* 1622 (1958); 20 (1959).

The negative ORD curve[18] of illudin S, $[\phi]_{589}$ −459°, $[\phi]_{375}$ −4,435° (25°, MeOH, $c = 0{\cdot}0069$) becomes positive in the isomer III, $[\phi]_{589}$ +499°, $[\phi]_{312}$ +21,380°, $[\phi]_{280}$ −22,440°, $[\phi]_{269}$ 0·00° (25°, MeOH, $c = 0{\cdot}0013$); these results together with other data will be reported in a separate paper.

Oxidation of isoilludin S (III) with chromic anhydride–pyridine at 50° yields the dihydrobenzofuran derivative (V), $\nu_{max}^{KBr}$ 3525, 1731, 1687 cm$^{-1}$, which gives a monoacetate (VI) having no hydroxyl groups (IR).

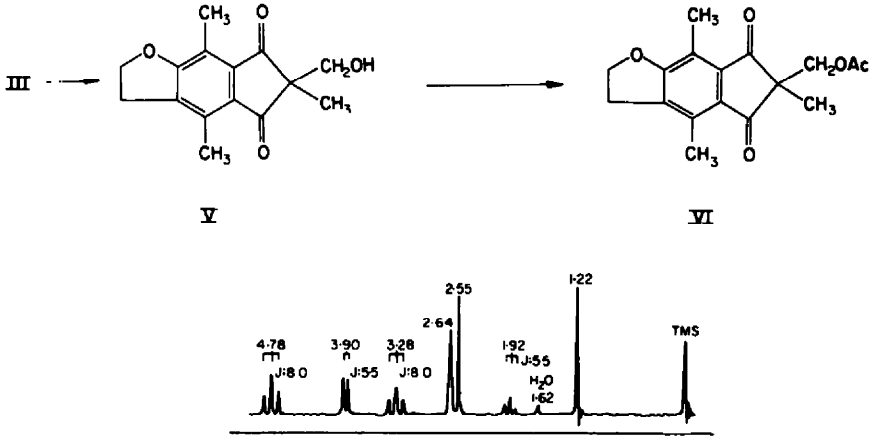



FIG. 2a. NMR spectrum of dihydrobenzofuran derivative V, dry $CDCl_3$, 100 mc.


FIG. 2b. NMR spectrum of dihydrobenzofuran derivative V after being shaken with $D_2O$, $CDCl_3$, 100 mc.

The structure of the oxidation product (V) can be established on grounds of spectral data. Fig. 2a shows the NMR spectrum of the oxidation product in dry $CDCl_3$ and Fig. 2b shows the spectrum of the same compound after being shaken with a little $D_2O$. The 3 methyl singlets and the pair of triplets are unchanged in both experiments, but a doublet at 3·90 ppm is reduced to a singlet which has the same chemical shift, and simultaneously a triplet at 1·92 ppm has disappeared. This can be accounted for by exchange of the hydroxyl proton with deuterium to remove the spin coupling (5·5 c/s) between methylene and hydroxyl protons.[19] This behaviour can be understood only when the oxidation product has a hydroxymethyl group attached to a quaternary carbon. The chemical shift of the pair of triplets each representing 2 protons corresponds to a benzylic methylene (3·28 ppm) and a methylene connected to

[18] ORD curves were measured with a Japan Spectroscopic Company ORD//UV5 spectropolarimeter.
[19] T. R. Govindchari, K. Nagarajan and H. Schmidt, *Helv. Chim. Acta* **46**, 433 (1963).

an oxygen (4·78 ppm). The 2 methylenes are spin coupled to each other and the signals do not change when shaken with $D_2O$. The coupling constant (J = 8·0 c/s) is distinctly larger than that encountered in aliphatic or saturated cyclic ethylenes which is usually 5–7 c/s, and corresponds to the coupling constant of a dihydrofuran derivative.[20] These facts indicate that the oxidation product (V) contains a dihydrobenzofuran moiety. Comparison of the UV spectra of the oxidation product with model compounds, indane-1,2-diones[21] and indan-1,3-diones[22] shows the oxidation product to be an indane-1,3-dione. The former group has only one absorption maximum, whereas the latter has 3 main absorptions. The oxidation product having 3 maxima, $\lambda_{max}^{EtOH}$ 256, 296, 330 m$\mu$ (log $\varepsilon$ 4·18, 3·44, 3·14) apparently is an indane-1,3-dione. The unusually low chemical shift for the 2 aromatic methyls (2·55 and 2·64 ppm) is accounted for by the magnetic anisotropy effect of peri carbonyls[23] of the indan-1,3-dione. The methyl resonating at 2·64 ppm and the triplet at 3·28 ppm which is due to the benzylic methylene, are somewhat broad. These signals become sharper upon double resonance experiment. It follows that the oxidation product clearly has structure V. The dihydrofuran moiety is derived from the spirocyclopropane in isoilludin S, and the presence of 2 aromatic methyls on the aromatic nucleus shows that the α-ketol moiety involving the tertiary hydroxyl is not located on the five-membered ring.

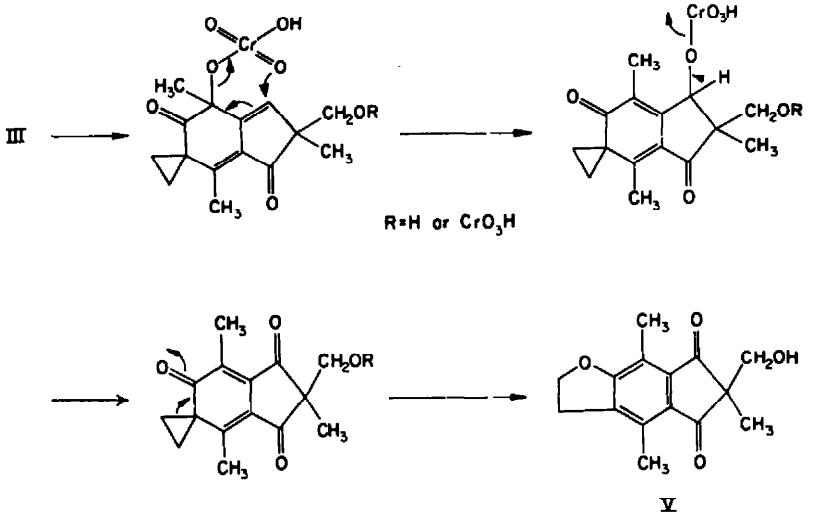


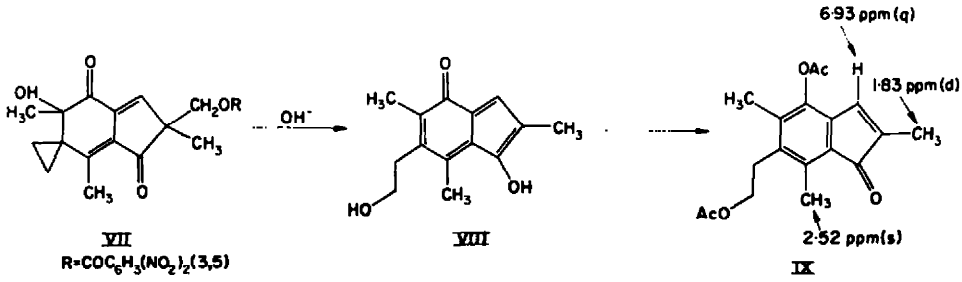

FIG. 3

The mechanism of this oxidation is presumably as shown in Fig. 3. The crowding of the tertiary chromic ester is relieved by allylic rearrangement of the ester group giving an *ortho*-quinonoid structure which gives the dihydrobenzofuran moiety by cleavage of the spirocyclopropane.

Chromic anhydride–pyridine oxidation of the mono-3,5-dinitrobenzoate of illudin

[20] *NMR Spectra Catalog* Spectrum No. 300. Varian Associates, Palo Alto, Calif. (1962).
[21] C. F. Koelsch and H. Hochmann. *J. Org. Chem.* **3**, 503 (1939).
[22] M. Carmack, M. B. Moore and M. E. Balis, *J. Amer. Chem. Soc.* **72**, 844 (1950).
[23] R. Harada, H. Kakisawa, M. Musya, K. Nakanishi and Y. Takahashi, *Tetrahedron Letters* No. 14, 603 (1962).

S (primary hydroxyl esterified) yields the diketo derivative (VII), which has only one hydroxyl band at 3511 $cm^{-1}$ (intramolecularly hydrogen-bonded tertiary hydroxyl, in dil. $CCl_4$). When the diketone VII is treated with alkali, the solution instantly turns red and affords a red pigment (VIII); $\nu_{max}^{KBr}$ 3300, 1681 $cm^{-1}$, which gives a yellow diacetate having both phenolic acetate bands (1759, 1210 $cm^{-1}$) and aliphatic acetate bands (1730, 1237 $cm^{-1}$). The ketonic carbonyl absorption (1703 $cm^{-1}$) in the diacetate IX is rather high for unsaturated ketones, but it is well known that cyclopentadienone derivatives show an absorption in the range for saturated ketones.[24]




VII
R=$COC_6H_3(NO_2)_2$(3,5)

VIII

IX

FIG. 4

Although a definite choice cannot be made between the tautomeric structures, the UV absorption of the red pigment (VIII) at 249, 374 and 440 m$\mu$ (log $\varepsilon$ 4·30, 3·43, 3·20) and its yellow acetate (IX) at 247, 344 and 400 m$\mu$ (log $\varepsilon$ 4·57, 3·36, 2·08) suggest the structures as shown in Fig. 4. The NMR spectrum of the diacetate is in full agreement with this structure. The transformation of diketone (VII) into the red

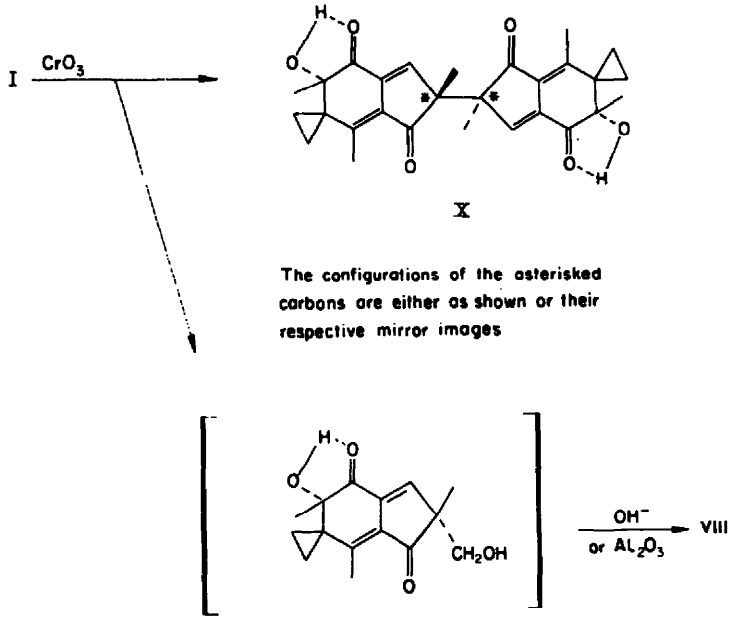



FIG. 5

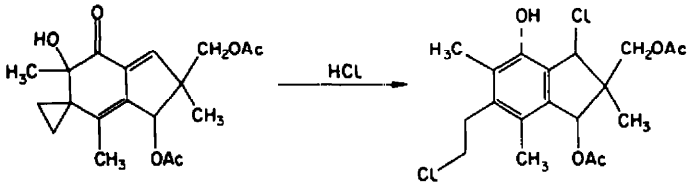
[24] E. D. Berhmann, *Progress in Organic Chemistry* (Edited by J. W. Cook) Vol. 3; p. 117. Butterworths, London (1955).

pigment involves hydrolysis of the ester followed by *retro*-aldol fission of the hydroxymethyl and transformation of the cyclopropane moiety. The red pigment (VIII) is also obtained by oxidation of illudin S itself with chromic anhydride-pyridine followed by passage through a column of alumina, or treatment with alkali. This oxidation affords the dimerized compound $C_{28}H_{30}O_6$ (X), as a by-product.

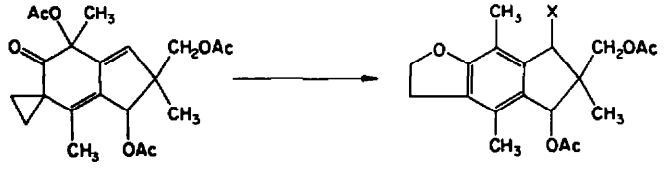
The structure of the dimer, m.p. > 180° (dec), is derived from spectroscopic data: the NMR spectrum shows 2 sets of methyls and vinyl signals as the dimer is not completely symmetrical. The cyclopropyl proton region remains relatively unaffected. The UV spectrum $\lambda_{max}^{EtOH}$ 302 m$\mu$ (log $\varepsilon$ 4·42) is similar to that which is obtained by subtracting the absorption of ethyl 3,5-dinitrobenzoate from that of the diketone (VII) excepting that the absorption is twice as intense. The IR spectrum has a cyclopropane band (3060 $cm^{-1}$) and strong bands at 1706, 1610 and 1596 $cm^{-1}$ due to the two s-*cis* $\alpha,\beta$-unsaturated ketone groups. Since the configurations at the carbon atoms bearing the t-hydroxyl groups remain unaltered this dimer can not be a meso-compound, and as excepted its ORD curve is negative with a trough at 403 m$\mu$: $[\phi]_{589}$ −758°, $[\phi]_{403}$ −2,710°, $[\phi]_{390}$ −2,100° (25°, dioxane, $c$ = 0·042). Although the configurations at the 2 termini carbons of the new bond is not clear, the doublet nature of the NMR spectrum suggests that the dimer either possesses no center of symmetry (as shown in X) or is a mixture of the two centrosymmetric structures (epimeric at one of the asterisked carbons shown in X). The formation of the dimer presumably occurs through a free radical intermediate.

Treatment of illudin S diacetate (III) with HCl gas in dry chloroform gave a phenolic compound XI, m.p. 109–111°, $\lambda_{max}^{EtOH}$ 292 m$\mu$; $\nu^{KBr}$ 3400, 1730, 1715, 1600; positive Beilstein test. NMR spectrum and thin layer chromatography, however, show that the product is a mixture of mainly 2 components possibly due to configurational difference of the entering chlorine atom.

Treatment of isoilludin S triacetate (IV) with various acids, affords a non-crystalline mixture having phenolic UV absorption; $\lambda_{max}^{EtOH}$ 291–295 m$\mu$. The spectrum, however, does not change in alkaline medium and hence it is presumably a dihydrobenzofuran derivative of the structure XII.



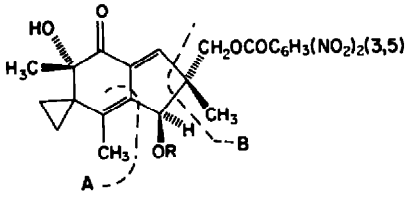

II XI




IV XII X=Cl, OTs, or OH

The relative configurations at the 3 asymmetric carbon atoms in illudin S (I) and isoilludin S (III) are identical because the former is converted into the latter by an acyloin rearrangement. In the following discussion the absolute configuration of the secondary hydroxyl group is deduced first, and then the configuration at each of the remaining optical centers relative to the secondary hydroxyl is determined.

The so-called "conformational dissymmetry rule",[25] which is an extension of Mills' rule[26] and the "benzoate rule",[27,25] was applied to the monoester (XIII) and diester (XIV) of illudin S in order to deduce the stereochemistry of the secondary hydroxyl group. As indicated in the Fig. a large positive shift of +622° takes place in going from the monoester to the diester. It is known that in cases where polarizability considerations (Mills' rule) and steric considerations (benzoate rule) lead to opposite absolute configurations, it is the former polarizability factor that exerts the predominating influence.[28]




XIII R = H, $[\phi]_D^{25}$ −405° (c = 0·1412, dioxane)

XIV R = $COC_6H_3(NO_2)_2(3,5)$, $[\phi]_D^{25}$ +217° (c = 0·1383, dioxane)

$\Delta[\phi]_D^{25} = +622°$

Molecular models suggest that of the 2 moieties A and B flanking the secondary hydroxyl group, the former with its peri methyl group can be taken as the more bulky. Although this might not be as clearcut as in other simple carbinols, there is a large difference in the polarizability of the 2 adjacent carbon atoms, the $sp^2$ carbon naturally being the more polar, and interpretation of the +622° shift in terms of Mills' rule leads to the absolute configuration shown.

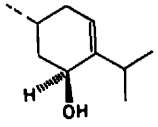
Horeau has recently developed an asymmetric synthesis that can be employed to determine the absolute configurations of a variety of secondary alcohols.[29] The method applied to the mono-3,5-dinitrobenzoate (XIII) of illudin S, led to an esterification yield of 66% and an optical yield of +33%. This indicates that moiety A is to

[25] J. H. Brewster, *Tetrahedron* **13**, 106 (1961).

[26] J. A. Mills, *J. Chem. Soc.* 4976 (1952).

[27] K. Freudenberg, *Stereochemie* p. 696. Deuticke, Leipzig (1933).

[28] For example the rotational shift of menth-4-en-3$\beta$-ol having the absolute configuration shown is



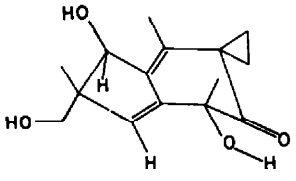

−310° (i.e., $[\phi]_D$ of alcohol −299°, $[\phi]_D$ of 3,5-dinitrobenzoate −609°). Mills' rule leads to the configuration indicated,[26] whereas the benzoate rule leads to the opposite configuration.

[29] A. Horeau, *Tetrahedron Letters* 506, 654 (1961); 965 (1962). Also private communciation. The authors are grateful to Professor Horeau for discussions at the occasion of the IUPAC Kyoto Symposium, April, 1964.

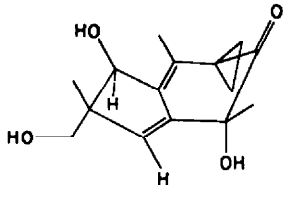
be taken as the group exerting the larger steric influence when the secondary hydroxyl is esterified.

The hydroxymethyl and secondary hydroxyl groups are considered to be in a *trans*-relationship since a detailed IR study of illudin S in the O—H stretching region does not show any evidence of intramolecular OH · · · OH bonding. Namely, a $10^{-3}$ mole solution of illudin S in $CCl_4$ has bands at 3636 (free—$CH_2OH$), 3615 (free—CHOH) and 3510 $cm^{-1}$ (C═O · · · H—O of α-ketol); at higher concentrations (in $CHCl_3$ solution) the intensity of the 2 free hydroxyl bands decreases and a broad band at 3500–3300 $cm^{-1}$ (associated OH) appears.

Molecular models of isoilludin S show that its six-membered ring can exist in 2 conformations; in one of them (XV) the t-hydroxyl is *quasi*-equatorial while in the other, (XVI), the methyl is *quasi*-equatorial. The IR spectrum of isoilludin S mono-3,5-dinitrobenzoate (primary OH esterified), m.p. 175°, in $CCl_4$ has a distinct band at




XV




XVI

3510 $cm^{-1}$ due to bonding of the t-hydroxyl to the carbonyl. Thus the t-hydroxyl is *quasi*-equatorial. The fact that the same sharp band persists in the solid state spectra of various monobenzoates of isoilludin S, i.e., *p*-nitro- (3490 $cm^{-1}$), *m*-nitro- (3480 $cm^{-1}$), *p*-bromo- (3490 $cm^{-1}$), and *p*-iodobenzoate (3480 $cm^{-1}$),[30] implies that the six-membered ring has the same conformation in both the dissolved and the solid state, and that the t-hydroxyl is *quasi*-equatorial. Although the X-ray study carried out by Professor Saito *et al.*[10] on the above mentioned *p*-iodobenzoate do not yet permit the differentiation between the hydroxyl and the methyl groups of the α-ketol moiety it clearly indicated that the *p*-iodobenzoate adopts conformation XV, i.e., the *quasi*-equatorial group is *cis* with respect to the *prim*-hydroxyl. This in conjunction with the IR evidence for an intramolecular hydrogen-bond leads to the full stereochemistry of illudin S (lampterol) and isoilludin S (isolampterol) as shown in formulae I and III, respectively.

The tertiary hydroxyl of isoilludin S is easily acetylated when treated with acetic anhydride and pyridine, and accordingly the possibility of an acyl migration from the primary to the tertiary hydroxyl group was considered (O · · · O distance is ca. 4 Å). However, this is not the case because when isoilludin S monobenzoate (primary hydroxyl converted to 3,5-dinitro- and *p*-bromo-benzoates) is further esterified to the

[30] The following two α-ketols also exhibited sharp absorption at 3480 $cm^{-1}$ (KBr):

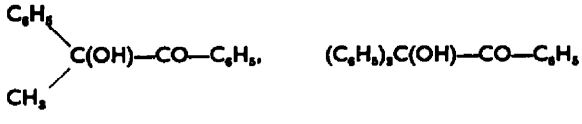
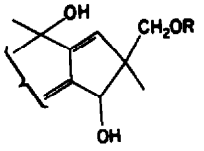


TABLE 2. CHEMICAL SHIFT OF —$CH_2$—OR IN ISOILLUDIN S MONOESTERS AND TRIESTERS

| | | |
|---|---|---|
|  | monobenzoate<br>R: $COC_6H_3(NO_2)_2$(3,5)<br>diacetate monobenzoate | <br>4·48 ppm (J: 10·0 c/s)<br>4·45 ppm (J: 11·0 c/s) |
| | monobenzoate<br>R: $COC_6H_4Br$(p)<br>diacetate monobenzoate | <br>4·12 ppm (J: 11·0 c/s)<br>4·18 ppm (J: 11·0 c/s) |

corresponding diacetate monobenzoates, the chemical shift of the esterified hydroxymethylene group (AB type quartet) remains practically the same. As shown in Table 2, the methylene group signals (AB type) appear at ca. 4·5 or 4·2 ppm, whereas in the triacetate it is located at the higher field of 3·97 ppm.

The carbon skeleton of illudin S could be derived biogenetically from a precursor of the humulene type (Fig. 6).

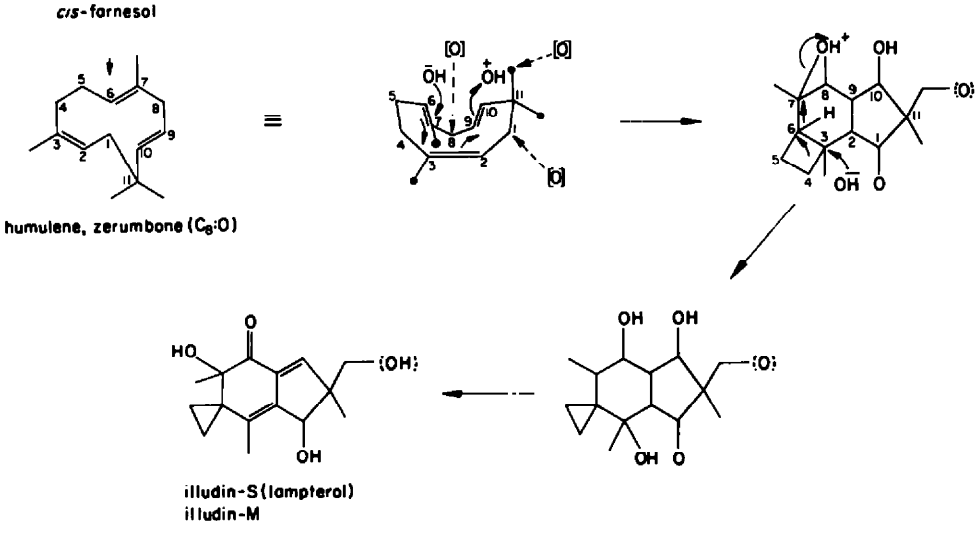



FIG. 6. Biogenesis of illudin S

## EXPERIMENTAL

M.ps were determined on a micro hot-stage and are uncorrected. The UV spectra were measured with a HITACHI EP-2 recording spectrometer. The IR spectra were measured with JASCO DS-301, IR-S, and HITACHI EPI-S models equipped with rock-salt prisms, while measurement of the hydroxyl absorption in dilute solution was carried out by a JASCO G-401 grating spectrometer in a 20 mm cell. The wavenumber was calibrated against the combination band of atmospheric vapour and $CO_2$ (3619 and 3631 $cm^{-1}$) or mica (3626 $cm^{-1}$). The NMR spectra were measured with a Varian A-60 (60 mc), Varian V-4300 (60 mc), Nihon Denshi JNM-C-60 (60 mc) and Varian HR-100 (100 mc) spectrometers. The chemical shifts are expressed in ppm using tetramethyl silane as internal reference.

*Isolation of illudin S (lampterol)*

Fresh mushroom (300 kg) was soaked in MeOH (100 l.) for 3 weeks, and the MeOH solution (270 l.) (containing large amount of water) was separated from the mycelium (88 kg) using a centrifugal separator. The mycelium was further soaked in MeOH (132 l.) for 3 days and centrifugal separation again gave the MeOH solution (152 l.). The combined MeOH solution was concentrated *in vacuo*

to 25 kg below 45°, and the residual syrup was treated with water (6 l.), and then with MeOH (100 l.) to remove mannitol. After addition of celite (1 kg) as a filter aid and agitation for 30 min, D-mannitol (9·4 kg) was removed from the methanolic solution (96 l.) using a centrifugal separator. The MeOH filtrate was again concentrated at 50° and the residue (15 l.) made up to 30 l. (aqueous solution) was defatted by 3 extractions with n-hexane (each 30 l.). The aqueous layer was extracted repeatedly (8 times) with the same volume of butyl acetate, and the combined butyl acetate extracts concentrated *in vacuo*. The residue (296 g) was dissolved in water (1·2 l.), and the insoluble oil separated, mixed with Sephadex G-25 (30 g), placed on top of a 2·5 × 5 cm Sephadex G-25 column, and eluted with water (2 l.). This eluate and the water soluble fraction were combined, passed through an Amberlite CG-45 (OH type) column (3 × 50 cm) to remove organic acids (mostly succinic acid), and the eluate concentrated *in vacuo* below 50°. The concentrate was extracted with ether (200 ml) 6 times, and the ether extract concentrated *in vacuo* to yield 32·5 g residue. Crystallization of the residue from acetone, followed by recrystallization from dichloroethane-acetone (10:2) afforded crystalline illudin S (lampterol; 6·2 g). m.p. 124–126°.

The second run from a batch of 347·5 kg of mushrooms afforded illudin S (15 g), yield $4 \times 10^{-4}$%.

*Illudin S* (I)

Illudin S is obtained as colourless needles; m.p. 124–126° from dichloroethane–acetone (10:2), positive to TTC (triphenyltetrazolium chloride) test. (Found: C, 67·62; H, 7·39; $C_{15}H_{20}O_4$ requires: C, 68·18; H, 7·63%.) $\lambda_{max}^{MeOH}$ 235, 320 mμ (log ε 4·10, 3·54); IR: (KBr) 3300 (broad), 1693, 1660, 1603, 1110, 1037 cm$^{-1}$; ($CHCl_3$) 3480, 3065, 1698, 1651, 1606, 1106, 1028 cm$^{-1}$; ($CCl_4$, $3 \times 10^{-3}$ M) 3636, 3615, 3510 cm$^{-1}$, (dioxane) 1700 cm$^{-1}$; NMR: see Table 1; mass: $M^+ = 264$.

Antitumour activity was 120 γ/kg as measured by the effect on Ehrlich mouse ascetic tumour, and the toxicity was 5 mg/kg on intraperitoneal injection into ordinary mice.

*Illudin S diacetate* (II).

To a solution of crude illudin S (3·0 g) in 15 ml pyridine, 30 ml acetic anhydride was added and the mixture allowed to stand at room temp overnight. The reaction mixture was poured on ice and stirred vigorously, when 2·2 g of crystals were obtained. Recrystallization first from pet. ether and then twice from ligroin gave illudin S diacetate; m.p. 102°. (Found: C, 65·74; H, 7·16; $C_{19}H_{24}O_6$ requires: C, 65·50; H, 6·94%.) $\lambda_{max}^{MeOH}$ 226, 245$^{infl.}$, 313 mμ (log ε 4·08, 4·02, 3·54): $\lambda_{max}^{isooctane}$ 226, 245, 314 mμ (log ε 4·10, 4·05, 3·54); IR: (KBr) 3540, 3072, 3048, 1740, 1731, 1698, 1666, 1603 (strong), 1107 cm$^{-1}$; ($CCl_4$) 3508, 3065, 1745, 1706, 1657, 1611 (strong), 1107 cm$^{-1}$; ($CCl_4$, $3 \times 10^{-3}$ M) 3508 cm$^{-1}$; NMR: ($CCl_4$) 6·43 (s, 1 H), 5·80 (s, 1 H), 3·99 (AB, $J_{AB} = 11{\cdot}4$ c/s, $\delta_{AB} = 0{\cdot}28$ ppm), 3·52 (s, 1 H, OH), 2·05, 1·97, 1·51, 1·37, 1·12 (s, each 3 H), 0·3–1·4 ppm (m, 4 H); mass: $M^+ = 348$.

*Potassium permanganate oxidation of illudin S*

*Formation of cyclopropane-1,1-dicarboxylic acid.* To a solution of 400 mg illudin S in 20 ml water was added dropwise 200 ml $KMnO_4$ (2·0 g) solution within a period of 12 hr while heating the reaction mixture on a water bath. Subsequently 12·0 g of powdered $KMnO_4$ was added in portions during a period of 48 hr, this time the reaction mixture being kept at 100–130° on an oil bath. After cooling, the reaction mixture was decanted and washed with hot water, and the combined solution slightly acidified with 2 N $H_2SO_4$. The solution was concentrated and extracted thrice with an equiv. volume of ether. Evaporation of the ether after drying ($Na_2SO_4$) gave crude hydroscopic crystals. These were submitted to silica chromatography and eluted with $CHCl_3$, upon which 15 mg of colourless needles were obtained.

The titration curve showed a two-stage dissociation and the NMR spectrum showed only 2 peaks, m.p. 134–135°; IR: (KBr) 3500, 2800–2400, 1715, 1653, 1611, 1422, 898 cm$^{-1}$; NMR: ($CDCl_3$) 1·34 (s), 5·52 (broad singlet); MW: (from titration) ca. 150.

This diacid was found to be cyclopropane-1,1-dicarboxylic acid by direct comparison with an authentic synthetic specimen.

*Isomerization of illudin S* (I) *into isoilludin S* (III)

(a) Illudin S (2 g) dissolved in ethyl acetate was repeatedly passed through the column of Brockmann's alumina (3 × 30 cm). Evaporation of the eluate gave crude isoilludin S contaminated with

a small quantity of starting material. Recrystallization from ethyl acetate afforded 1·2 g isoilludin S; m.p. 179–180°. (Found: C, 68·34; H, 7·57; $C_{15}H_{20}O_4$ requires: C, 68·18; H, 7·68%.) $\lambda_{max}^{EtOH}$ 252 mμ (log ε 4·31); IR: (KBr) 3500 (broad), 3065, 3038, 1697, 1645 $cm^{-1}$; (dioxane) 1714 $cm^{-1}$, NMR: ($CDCl_3$) 5·69 (s, 1 H), 4·60 (s, 1 H), 3·40 (s, 2 H), 2·22 (s, 3 H, OH), 1·67, 1·49, 1·17 (s, each 3 H); mass: $M^+ = 264$.

(b) When illudin S was heated on a hot-stage at 200°, there was obtained a small amount of isoilludin S as the sublimate; the UV spectrum of the residue on the hot-stage was also that of isoilludin S.

*Isoilludin S triacetate* (IV)

Isoilludin S(120 mg) in 0·5 ml pyridine was treated with 1 ml acetic anhydride and this was allowed to stand at room temp overnight. The reaction mixture was poured on ice, when stirring and rubbing of the beaker wall with a spatula gave isoilludin S triacetate. Recrystallization from ligroin gave 50 mg pure acetate; m.p. 112–113°; (Found: C, 64·68; 6·87; $C_{21}H_{26}O_7$ requires: C, 64·60; H, 6·71%). $\lambda_{max}^{EtOH}$ 252 mμ (log ε 4·30), IR: (KBr) 1741, 1731, 1708, 1663, 1088, 1044 $cm^{-1}$; ($CCl_4$) 3027, 1742, 1717, 1665 $cm^{-1}$; NMR: ($CDCl_3$) 5·90 (s, 1 H), 5·42 (s, 1 H), 3·99 (AB, $J_{AB} = 11·4$ c/s, $\delta_{AB} = 0·31$ ppm), 2·09, 2·01, 1·59, 1·49, 0·99 (s, each 3 H), 1·1–1·8 (m, 4 H); mass $M^+ = 390$.

*Determination of periodate oxidation equivalent*

(a) *Illudin S.* Illudin S (25 mg) in 5 ml water was treated with 3 ml of 0·1 N $HIO_4$ and 2 ml water. One ml aliquots of the reaction mixture were pipetted out at suitable intervals, and treated with 2 ml $NaHCO_3$ aq, 2 ml 0·1 N $Na_3AsO_3$ and 1 ml of 20% KI aq. The mixture was kept at room temp for 15 min in the dark, and the excess $Na_3AsO_3$ was titrated with 0·1 N I solution ($f = 1·0036$). Illudin S consumed one mole of periodic acid.

(b) *Isoilludin S.* The determination was carried out in aqueous 0·1 N $HIO_4$. Isoilludin S consumed one mole of periodic acid.

(c) *Illudin S diacetate.* The determination was carried out in a 50% water–MeOH containing 0·1 N $HIO_4$. It also consumed one mole of periodic acid.

*Periodate oxidation of illudin S*

Illudin S (100 mg) was dissolved in 2 ml aqueous 0·5 N $NaIO_4$ and allowed to stand at 30° for 24 hr. After addition of NaCl to saturation point, the oxidation product was extracted with ethyl acetate 3 times. The combined extracts were evaporated *in vacuo* to dryness without any washing, because the oxidation product is easily soluble in water, and 117 mg syrup were obtained. The oxidation product (60 mg) was purified by silica chromatography, and eluted with $CHCl_3$ and ethyl acetate. The oxidation product was eluted in the early fractions of ethyl acetate, but its crystallization from several solvents was unsuccessful. Thin layer chromatography (silica–ethyl acetate) gave 2 spots, one being much smaller than the other. The oxidation product was positive to iodoform test; $\lambda_{max}^{EtOH}$ 259 mμ; IR: ($CHCl_3$) 2800–2400, 1720 (carboxyl), 1700 $cm^{-1}$; *pKa* = 4·6 (in water).

*Periodate oxidation of isoilludin S*

To 5 ml aqueous 1 N $HIO_4$ was added 300 mg isoilludin S and the reaction mixture allowed to stand at room temp for 1 hr. The oxidation product was extracted with ethyl acetate after addition of NaCl. Evaporation of the solvent and purification of the $CHCl_3$ soluble part (180 mg) by silica chromatography did not give crystalline material, but its thin layer chromatgram showed that the syrup was quite pure. The oxidation product gave a positive iodoform test; $\lambda_{max}^{EtOH}$ 230, 278 mμ; IR: (KBr) 2800–2400, 1720 (carboxyl), 1685 $cm^{-1}$ (αβ-unsaturated ketone).

*Chromic anhydride–pyridine oxidation of isoilludin S* (V)

To a $CrO_3$–pyridine complex ($CrO_3$ 450 mg, pyridine 6 ml) was added 200 mg isoilludin S in 3 ml pyridine with cooling at 0°, and the mixture allowed to stand at 50° for 24 hr. The reaction mixture was filtered and the dark brown solid washed with pyridine. The combined pyridine solutions were diluted with water and extracted with ethyl acetate. After drying ($Na_2SO_4$), the solvent was evaporated *in vacuo* to dryness, when crude crystals (100 mg) deposited. Purification by silica chromatography, elution with $CHCl_3$, and recrystallization from EtOH gave 30 mg of the dihydrobenzofuran derivative

V; m.p. 198–199°. (Found: C, 69·13; H, 6·23; $C_{15}H_{16}O_4$ requires: C, 69·21: H, 6·20%.) $\lambda_{max}^{EtOH}$ 256, 296, 330 mμ (log ε 4·14, 3·44, 3·14); IR: (KBr) 3525, 1731, 1687, 1573, 1303, 1048 cm$^{-1}$; NMR: ($CDCl_3$) 4·78 (t, J = 8·0 c/s, 2H), 3·90 (d, J = 5·5. c/s, 2 H), 3·28 (t, J = 8·0 c/s, 2 H), 2·64, 2·55 (s, each 3 H), 1·92 (t, J = 5·5 c/s, hydroxyl), 1·22 (s, 3 H).

Acetylation with acetic anhydride pyridine yielded the monoacetate VI, m.p. 121° (n-hexane) $\lambda_{max}^{EtOH}$ 258, 299, 330 mμ; IR: (KBr) 1745, 1730, 1692, 1574, 1319, 1242, 1038 cm$^{-1}$; ($CCl_4$) 1756, 1742, 1705, 1581, 1312, 1235, 1043 cm$^{-1}$; mass: $M^+$ = 302 ($C_{17}H_{18}O_6$).

*Illudin S mono-(3,5-dinitro)-benzoate* (XIII)

To a solution of illudin S (200 mg) in 2 ml pyridine, 220 mg 3,5-dinitrobenzoyl chloride was added and the reaction mixture allowed to stand overnight at room temp. It was then poured onto ice-water and the precipitates separated by filtration. Recrystallization from $CHCl_3$ gave pale yellow needles (132 mg) of illudin S mono-(3,5-dinitro)-benzoate; m.p. 177–178°. (Found: C, 58·03; H, 4·95; N, 6·33; $C_{22}H_{22}O_9N_2$ requires: C, 57·63; H, 4·84: N, 6·11%.) IR: (KBr) 3490, 1713, 1615, 1557, 1360 cm$^{-1}$; ($CHCl_3$) 3480, 1739, 1702, 1609, 1548, 1345 cm$^{-1}$; ($CCl_4$, 3 × 10$^{-3}$ M) 3613, 3530; NMR ($CDCl_3$) 9·20 (q, 1 H), 9·05 (d, 2 H), 6·50 (s, 1 H), 4·75 (s, 1 H), 4·31 (s, 2 H), 1·91 (s, 3 H), 1·30 (s, 6 H), 0·8 – 1·1 ppm (m, 4 H), $[\phi]_D^{25}$ = −404·6° (c = 0·1419, dioxane).

*Diketoilludin S mono-(3,5-dinitro)-benzoate* (VII)

To a solution of the illudin S mono-(3,5-dinitro)-benzoate (200 mg) in 2 ml pyridine, $CrO_3$–pyridine complex ($CrO_3$ 200 mg, pyridine 1 ml) was added with cooling in an ice-bath, and the mixture allowed to stand at room temp overnight. The reaction mixture was filtered and the dark brown solid washed with pyridine. The combined solution was diluted with water and extracted with ethyl acetate. The extract was washed successively with $H_2SO_4$ sat. $NaHCO_3$ aq and sat. NaCl aq. Evaporation of the solvent *in vacuo* after drying ($Na_2SO_4$) gave a syrup, which was dried in a dessicator over $P_2O_5$ at red. press. Addition of a few drops $CHCl_3$ and rubbing the wall of the flask with a spatula led to crystallization. Recrystallization twice from EtOH gave 112 mg of white diketoilludin S mono-(3,5-dinitro)-benzoate; m.p. 175–176°. (Found: C, 58·08; H, 4·38; N, 6·36; $C_{22}H_{20}O_9N_2$ requires: C, 57·89; H, 4·42; N, 6·14%). $\lambda_{max}^{EtOH}$ 220$^{end}$, 294 mμ (log ε 4·36, 4·09); IR: (KBr) 3460, 1743, 1726, 1709, 1606, 1548, 1345 cm$^{-1}$; ($CHCl_3$) 3500, 1743, 1713, 1603, 1550, 1342 cm$^{-1}$; ($CCl_4$, 3 × 10$^{-3}$ M) 3511 cm$^{-1}$; NMR: ($CDCl_3$) 9·10 (q, 1 H), 8·90 (d, 2 H), 5·84 (s, 1 H), 4·62 (AB, $J_{AB}$ = 11·0 c/s, $\delta_{AB}$ = 0·23 ppm), 2·10, 1·35, 1·32 ppm (s, each 3 H).

*Red pigment* VIII

(a) *From diketoilludin S mono-(3,5-dinitro)-benzoate.* Diketoilludin S mono-(3,5-dinitro)-benzoate (100 mg) in 10 ml dioxane was treated with 10 ml 1 N NaOH aq, when the mixture turned purple red. The reaction mixture was allowed to stand overnight at room temp. After addition of 1 N $H_2SO_4$, the solution was extracted with ether in a Soxhlet extractor and the extract washed with $NaHCO_3$ aq and sat. NaCl aq. Evaporation of the ether after drying ($Na_2SO_4$) gave red crystals. Recrystallization from ethyl acetate–EtOH gave 10 mg pure pigment; m.p. 207–209°. (Found: C, 72·23; H, 7·31; $C_{14}H_{16}O_2$ requires: C, 72·39; H, 6·94%.) $\lambda_{max}^{EtOH}$ 249, 374, 440 mμ (log ε 4·30, 3·43, 3·20); IR: (KBr) 3360, 1681, 1603, 1240, 1024 cm$^{-1}$; mass: $M^+$ = 232 ($C_{14}H_{16}O_3$).

(b) *From the oxidation product of illudin S.* The syrup (370 mg) resulting from the $CrO_3$–pyridine oxidation of illudin S was dissolved in 20 ml 1 N alcoholic KOH, when the solution instantly turned deep red. The reaction mixture was allowed to stand at room temp for 2 hr, then acidified with 1 N $H_2SO_4$. The acidified solution was concentrated and diluted with water, and extracted with ether in a Soxhlet extractor. Evaporation of ether after washing with sat. NaCl aq and drying ($Na_2SO_4$) gave crude red crystals. Purification by silica chromatography and elution with $CHCl_3$, followed by recrystallization from ethyl acetate–EtOH gave 22 mg red pigment VIII.

(c) *From the oxidation product of illudin S.* The syrup (430 mg) obtained by $CrO_3$–pyridine oxidation of illudin S was placed on a column of alumina when the column turned gradually orange. Elution with ether, ethyl acetate and EtOH gave 18 mg red crystals from the ethyl acetate fraction. The amorphous product from the EtOH fraction was again submitted to silica chromatography and eluted with $CHCl_3$, when a small quantity of a red crystal was obtained. Recrystallization of the crystals afforded a small quantity of the red pigment VIII.

*Acetylation of the red pigment* IX

The red pigment was treated with acetic anhydride–pyridine yielding the acetate in quantitative yield. Recrystallization from EtOH gave yellow needles of the acetate IX; m.p. 147–148°. (Found: C, 68·55; H, 6·31; $C_{18}H_{20}O_5$ requires: C, 68·34; H, 6·37%). $\lambda_{max}^{EtOH}$ 247, 344, 400 mμ (log ε 4·57, 3·36, 2·08); IR: (KBr) 1759, 1730, 1703, 1603, 1237, 1210 cm$^{-1}$; NMR: ($CDCl_3$) 6·93 (q, J = 2·0 c/s, 1 H), 4·09 (t, J = 7·5 c/s, 2 H), 2·97 (t, J = 7·5 c/s, 2 H), 2·52, 2·38, 2·13, 2·06 (s, each 3 H), 1·83 ppm (d, J = 2·0 c/s, 3 H).

*Dimer* X—*Chromic anhydride oxidation of illudin S*

Illudin S (500 mg) in 10 ml pyridine was treated with $CrO_3$–pyridine complex ($CrO_3$ 1 g, pyridine 10 ml) at room temp overnight. The reaction mixture was poured on ice and the brown mass separated by filtration and washed with pyridine. The combined filtrate was diluted with water and extracted with ethyl acetate 4 times. Drying ($Na_2SO_4$) and evaporation of the solvent *in vacuo* deposited white crystals. Washing the crystals with $CHCl_3$ and recrystallization from ethyl acetate gave 56 mg dimer (less yield in other runs); m.p. 180° (darkening very slowly from ca. 180°). (Found: C, 72·45; H, 6·59; $C_{28}H_{30}O_6$ requires: C, 72·71; H, 6·51%.) $\lambda_{max}^{EtOH}$ 302 mμ (log ε 4·42); IR: (KBr) 3480, 3060, 1706, 1610, 1596 cm$^{-1}$; NMR: ($CDCl_3$) 7·16, 7·05 (s, 1 H), 3·59 (s, 2 H, hydroxyl), 2·07, 2·02 (s, 3 H), 1·35, 1·31 (s, 3 H), 1·20, 1·12 (s, 3 H), 1·3–0·1 ppm (m, 8 H); mass: a dimer was suggested by the peaks appearing higher than the monomeric $M^+$ peak.

*Acid treatment of illudin S diacetate* (XI)

Illudin S diacetate (300 mg) in 10 ml $CHCl_3$ was treated with dry HCl gas for 3 hr during which the solution was cooled in an ice-bath. Evaporation of the solvent afforded a syrup, which crystallized upon cooling. Recrystallization of the crude crystals twice from $CCl_4$ gave white crystals (60 mg); m.p. 109–111°; $\lambda_{max}^{EtOH}$ 292 mμ, IR: (KBr) 3400, 1730, 1715, 1600, 1250; Beilstein test: positive; thin layer chromatography showed the product to be a mixture of mainly two components.

*Application of Horeau's asymmetric synthesis to illudin S mono*(3,5-*dinitro*)-*benzoate*

To 24·3 mg (0·0531 m mole) illudin S-mono-3,5-dinitrobenzoate was added 154 mg pyridine and 53·6 mg α-phenylbutyric anhydride. After allowing to stand at room temp for 15 hr, a small drop of water was added and the mixture kept at 100° for 30 min, poured into a separatory funnel containing 30 ml benzene and 30 ml water, and titrated in the presence of phenolphthalein with 0·1 N NaOH; 3·09 cc of 0·1 N NaOH was consumed corresponding to an esterification yield of 66%.

The acidified aqueous phase was extracted with benzene and the benzene layer was then concentrated to 1 cc.

Observed rotation in a 0·5 dm tube: +0·096° (D line); optical yield, 33·4%.

*Illudin S di*-(3,5-*dinitro*)-*benzoate* (XIV)

A mixture of illudin S (200 mg) and 3,5-dinitrobenzoyl chloride (400 mg) was allowed to stand for 24 hr at room temp. The reaction mixture was diluted with ethyl acetate (50 ml), and the solution washed successively with 5% $H_2SO_4$, water, sat. $NaHCO_3$ aq. water and sat. NaCl aq, and evaporated to dryness *in vacuo*. It gave 388 mg residue and recrystallization from ethyl acetate–EtOH (10:2) afforded 120 mg crystalline di-3,5-dinitrobenzoate of illudin S; m.p. 167°. (Found: C, 53·53; H, 4·14; N, 8·34; $C_{28}H_{24}O_4$ requires: C, 53·37; H, 3·68; N, 8·59). IR: (Nujol) 3480, 1731, 1696, 1655, 1627, 1603, 1550; $[\phi]_D^{25°} = +217·1°$ ($c$ = 0·1383, dioxane).

*Isoilludin S mono*-(3,5-*dinitro*)-*benzoate*

Similarly, isoilludin S (200 mg) and 200 mg of 3,5-dinitrobenzoyl chloride in 5 ml pyridine afforded 70 mg monoester after recrystallization from $CCl_4$–$CHCl_3$ (4:1); m.p. 175°. (Found: C, 57·41; H, 4·56; N, 6·06; $C_{28}H_{22}O_9N_2$ requires: C, 57·64; H, 4·84; N, 6·11%). IR: (KBr) 3520, 1730, 1707, 1660, 1550, 1347 cm$^{-1}$; ($CHCl_3$) 3620, 3540, 1740, 1709, 1660, 1550, 1345 cm$^{-1}$; ($CCl_4$, 3 × 10$^{-3}$ M) 3613, 3530 cm$^{-1}$; NMR: ($CDCl_3$) 5·73 (s, 1 H), 4·70 (s, 1 H), 4·48, (AB, $J_{AB}$ = 10·0 c/s, $\delta_{AB}$ = 0·23 ppm), 2·35 (s, broad, 2 H, hydroxyl), 1·70, 1·46, 1·33 (s, each 3 H), 1·0–1·5 ppm (m, 4 H).

*Acetylation of isoilludin S mono*-(3,5-*dinitro*)-*benzoate*

Isoilludin S mono-(3,5-diniteo)-benzoate (40 mg) afforded 26 mg diacetate with acetic anhydride–pyridine; m.p. 144–146° (ligroin). (Found: C, 57·95; H, 4·81; N, 4·84; $C_{32}H_{28}O_{11}N_2$ requires: C,

57·56; H, 4·83; N, 5·16%). IR: (KBr) 1733, 1710, 1666, 1550, 1345 $cm^{-1}$; NMR: ($CDCl_3$) 6·18 (s, 1 H), 5·43 (s, 1 H), 4·45 (AB, $J_{AB}$ = 11·0 c/s, $\delta_{AB}$ = 0·48 ppm), 2·20, 1·95, 1·67, 1·48, 1·15 (s, each 3 H), 0·8–1·8 ppm (m, 4 H).

*Isoilludin S mono-*p*-bromobenzoate*

Isoilludin S (500 mg) yielded 475 mg mono-*p*-bromobenzoate with *p*-bromobenzoyl chloride–pyridine, m.p. 154° (n-hexane–$CHCl_3$, 10:1). (Found: C, 59·06; H, 5·17; $C_{22}H_{23}O_5Br$ requires: C, 59·07; H, 5·15%.) IR: (KBr) 3490, 1710, 1700 sh, 1645, 1590, 1480 $cm^{-1}$; NMR: ($CDCl_3$) 7·62 (AB, $J_{AB}$ = 9·0 c/s, $\delta_{AB}$ = 0·24 ppm), 5·79 (s, 1 H), 4·59 (s, 1 H), 4·12 (AB, $J_{AB}$ = 11·0 c/s, $\delta_{AB}$ = 0·24 ppm), 3·60 (s, broad, hydroxyl), 1·60, 1·51, 1·29 (s, each 3 H), 1·0–1·8 ppm (m, 4 H).

*Acetylation of isoilludin S mono-*p*-bromobenzoate*

Acetylation of isoilludin S mono-*p*-bromobenzoate (200 mg) with acetic anhydride and pyridine gave 250 mg syrup showing only a single spot on TLC (silica–$CHCl_3$); IR: ($CCl_4$) 1728, 1660, 1590, 1485 $cm^{-1}$; NMR: ($CDCl_3$) 7·69 (AB, $J_{AB}$ = 8·5 c/s, $\delta_{AB}$ = 0·36 ppm), 5·97 (s, 1 H), 5·46 (s, 1 H) 4·18 (AB, $J_{AB}$ = 11·0 c/s, $\delta_{AB}$ = 0·36 ppm), 2·09, 1·95, 1·55, 1·48, 1·12 (s, each 3 H), 1·12–1·17 ppm, (broad, 4 H).

*Isoilludin S mono-*p*-iodobenzoate for X-ray analysis*

To 100 mg isoilludin S in 100 ml dioxane was added 200 mg *p*-iodobenzoyl chloride in 1 ml pyridine and the reaction mixture refluxed for 6 hr. Ether was added to the cooled mixture which was then washed successively with 1 N $H_2SO_4$, sat. $NaHCO_3$ aq and water. Evaporation of the organic solvent after drying ($Na_2SO_4$) gave the crude benzoate, which was recrystallized from ether–ligroin (1:1) to colourless needles; m.p. 147–148°; IR: (KBr) 3480, 1710, 1680, 1645, 1577, 1288, 1106 $cm^{-1}$; NMR: ($CDCl_3$) 7·70 (AB, $J_{AB}$ = 8·5 c/s, $\delta_{AB}$ = 0·18 ppm), 5·79 (s, 1 H), 4·59 (s, 1 H), 4·13 (AB, $J_{AB}$ = 8·5 c/s, $\delta_{AB}$ = 0·23 ppm), 3·60 (broad, hydroxyl), 1·71, 1·50, 1·29 (s, each 3 H), 1·9–1·1 ppm (m, 4 H).

The large crystals for X-ray analysis were prepared by allowing an ether solution of the benzoate to evaporate at room temp and picking out the needles.

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