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# STUDIES ON JULIMYCINS—II

## THE STEREOCHEMISTRY OF JULIMYCIN B-II

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**Abstract**—The configuration and conformation of julimycin B-II have been elucidated by spectroscopic examination and chemical study. The absolute configuration inferred from optical properties of the degraded compounds is described.

IN THE previous paper,[1] the structure of julimycin B-II, a new anti-polio antibiotic, was established as formula I. This paper elucidates the stereochemistry of julimycin B-II.

As stated, julimycin B-II consists of two identical moieties and the conformation of each unit should be the same since the NMR spectrum indicates the symmetrical nature of the molecule.[1]

Julimycin B-II is optically active,[2] and the experiments shown in Chart 1 prove that the optical activity of I is not due to the restricted rotation about the 7–7′ linking, but is due to the asymmetric C atoms (starred) in the molecule. Mild dehydration[1] of I gave the optically active bisanhydro derivative II ($[\alpha]_D^{23}$ −108·2°, $CHCl_3$), but hydrogenolysis of II resulted in an optically inactive compound III (by ORD). Accordingly, the absolute configuration of each unit of I must be the same, otherwise

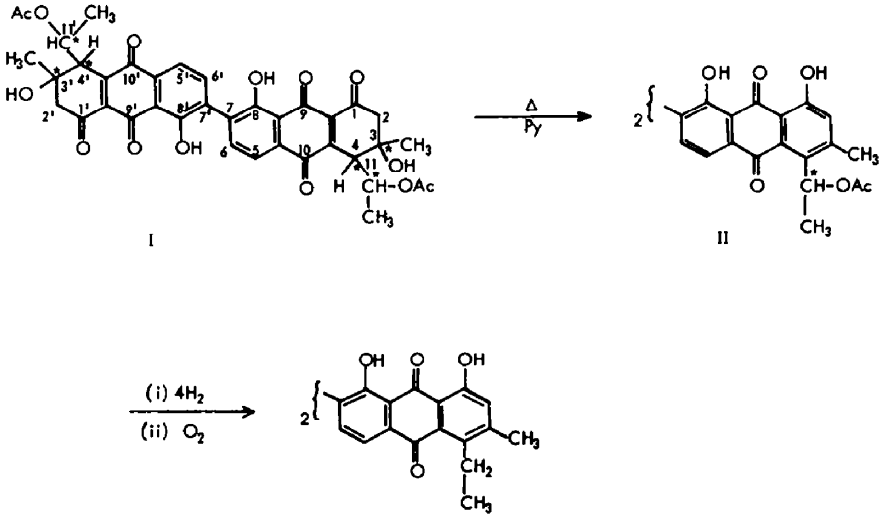
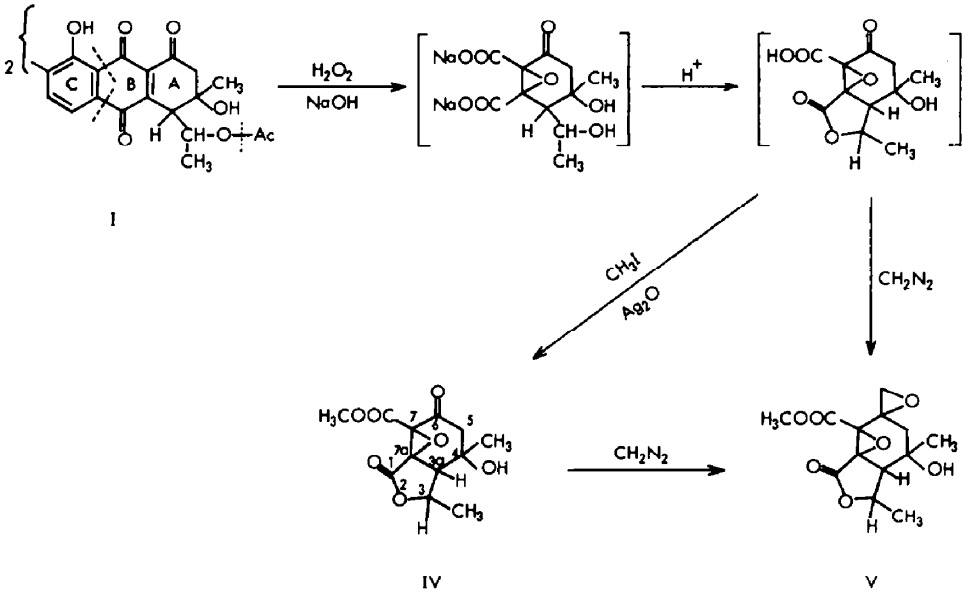


CHART 1

the whole molecule would have symmetrical conformations (meso form) and then could not be optically active.

In order to confirm the above assumption, I was oxidized with hydrogen peroxide in the presence of alkali. Treatment of the crude acid with methyl iodide–silver oxide and with diazomethane gave compounds IV and V, respectively.



I IV V

CHART 2

The compound IV, $C_{11}H_{11}O_6 \cdot OMe$, lacks an aromatic part and an α,β-unsaturated ketone (UV spectrum) and its IR spectrum indicates the presence of an OH band (3428 $cm^{-1}$), a γ-lactone band (1768 $cm^{-1}$), and additional two CO bands (1756 $cm^{-1}$, 1726 $cm^{-1}$). The OH group seems to be tertiary, because on treatment IV with acetic anhydride–pyridine, the unchanged material was recovered. This evidence suggests that epoxidation at the A/B juncture and fission at the dotted lines (cf. Chart 2) occurred during oxidation. This is supported by the IR band at 1756 $cm^{-1}$ which shows an abnormally high frequency shift characteristic of the α,β-epoxy ester.[3] Further, the assignment of the NMR spectrum* shown in Fig. 1 is in accord with the structure IV.

On the other hand, the IR spectrum of the product V confirms the presence of all functional groups present in IV except the 6-membered ring ketone. Its analysis suggests the molecular formula $C_{12}H_{13}O_6 \cdot OMe$ which shows the introduction of one $CH_2$ unit into IV. From these results, it was assumed that diazomethane attacks not only the carboxyl group but also the ketone group to give a spiro epoxy ester V. In fact, treatment of IV with diazomethane in methanol gave V in excellent yield. The NMR spectrum which shows the signals of $CH_2$ on a spiro epoxide ring (at δ 3·02 and 3·30, cf. Fig. 2) agrees with structure V.

* NMR spectra were taken with a Varian A-60 spectrometer unless otherwise stated. Chemical shifts are expressed in δ (ppm) downfield from TMS used as internal reference.

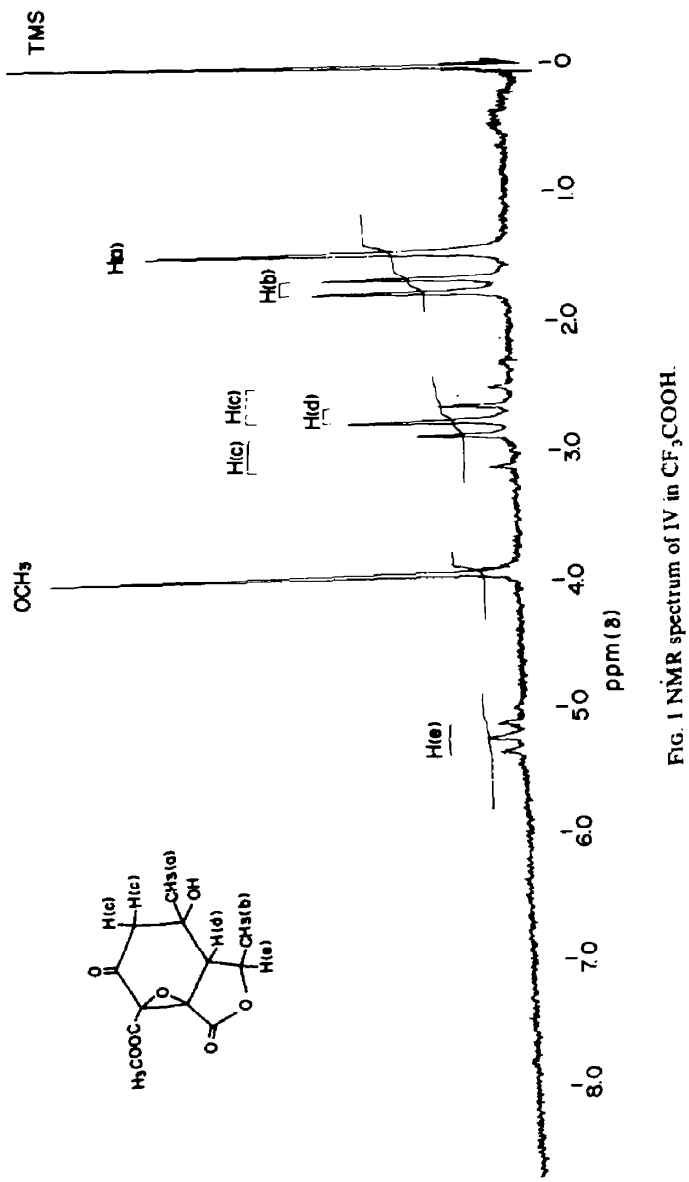



FIG. 1 NMR spectrum of IV in $CF_3COOH$.

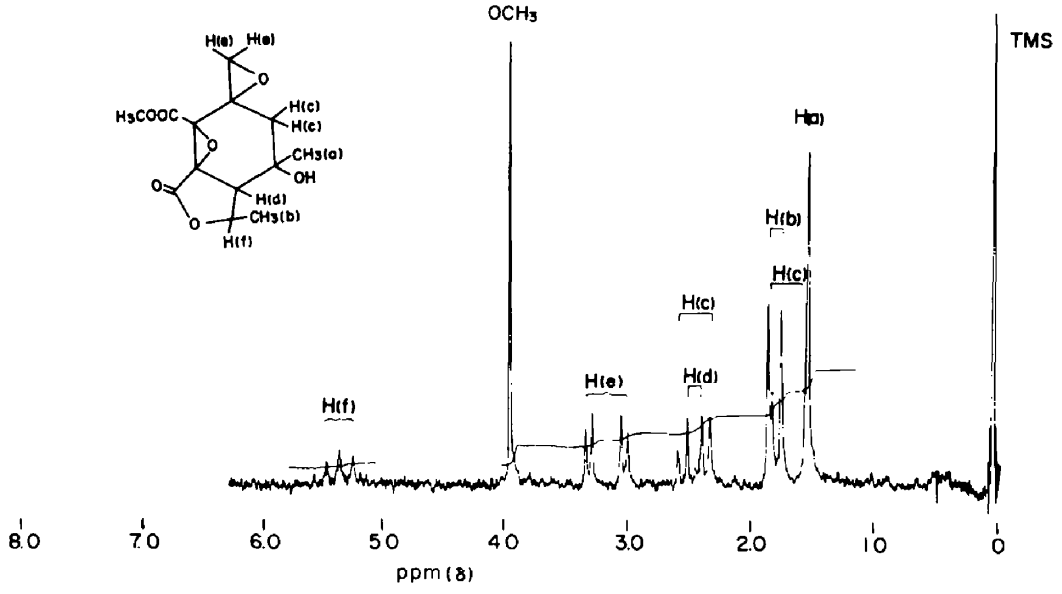



FIG. 2 NMR spectrum of V in $CF_3COOH$.

Both products, IV and V, resulting from a Dakin-type fission,[4] include all the asymmetric C atoms of I, and they clearly show optical activity, which proves the assumption that the two units of the original compound have the same absolute configuration.

Careful examination of the NMR spectra of julimycin B-II acetates[1] [8,8'-O-diacetate (VI), 3,3'-O-diacetate (VII) and 3,3',8,8'-O-tetraacetate (VIII)] revealed that the proton resonances of $C_2$—$H_2$, $C_3$—$CH_3$, and $C_4$—H always appear as broad signals suggesting the presence of long-range couplings between them. This was confirmed by the double resonance experiments of VII at 100 Mc. The assignment of the proton signals and the results of the spin decoupling experiments are shown in Fig. 3.

Double irradiation at the $C_4$—$H_{(e)}$ frequency sharpened the $C_2$—$H_{(d)}$ signal without changing other signals except the $C_{11}$—$H_{(f)}$ signal which was reduced to a quartet. Subsequently, the double irradiation at the frequency of $C_3$—$CH_{3(b)}$ resonance resulted in sharpening the $C_2$—$H_{(c)}$ signal. These results are explained by the presence of the respective long-range couplings between $C_4$—$H_{(e)}$ and $C_2$—$H_{(d)}$; and between $C_3$—$CH_{3(b)}$ and $C_2$—$H_{(c)}$, which according to the "W-letter rule"[5] suggest an equatorial $C_4$—$H_{(e)}$ and an axial $C_3$—$CH_{3(b)}$ group. Consequently, the configuration and the conformation of the substituents at $C_3$ and $C_4$ must be as illustrated in Fig. 3.* The axial orientation of the large groups at $C_3$ and $C_4$ is favoured probably owing to the steric hindrance of the $C_4$-group to the CO function at $C_{10}$ and the substituents at $C_3$.

In order to ascertain the configuration at $C_{11}$, the conformation of the $C_4$-side-

* The enantiomer which has β-Me group at $C_3$ is shown tentatively. The absolute configuration will be discussed below.

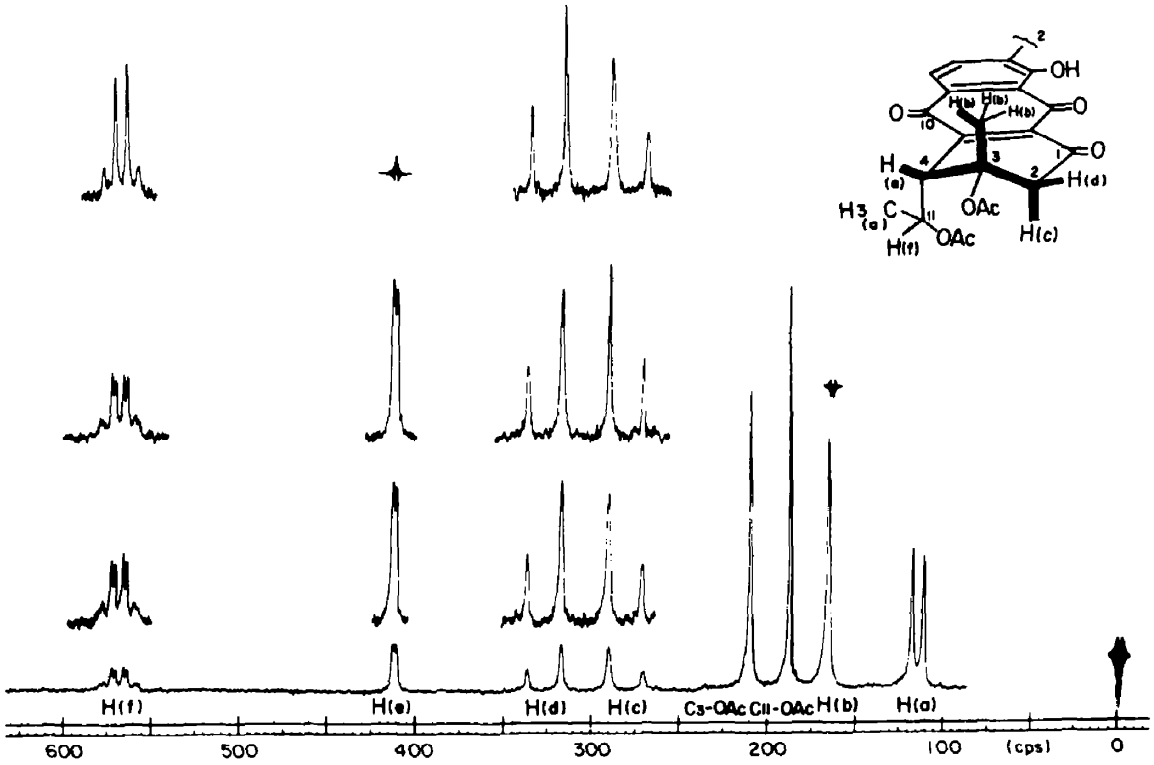
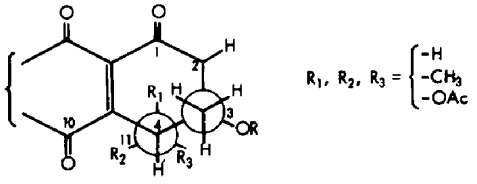



FIG. 3 NMR spectrum of VII and frequency-swept-decoupling experiments (in $CDCl_3$).

chain† was inspected spectroscopically. Fig. 4 indicates the hydroaromatic ring in the Newman projection.

In the NMR spectra, the signal of $C_{11}$—H is observed at a considerably lower field (cf. Table 1) probably owing to the anisotropic effects of some neighbouring functions. Moreover, the coupling constant ($J_{4,11}$ = 2·5 c/s, in all cases) suggests that the dihedral angle between $C_4$—H and $C_{11}$—H is approximately 60°, and not 180°.[6] From these facts, it is plain that $R_1$ in Fig. 4 cannot be hydrogen.



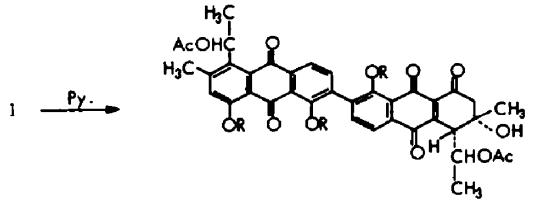

I, VI: R = H
VII, VIII: R = Ac

FIG. 4

TABLE 1. THE CHEMICAL SHIFTS OF $C_{11}$—H, $C_{11}$—$CH_3$ AND $C_3$—$CH_3$ OF JULIMYCIN B-II ACETATES (IN $CDCl_3$)

| | $C_{11}$—H | $C_{11}$—$CH_3$ | $C_3$—$CH_3$ |
|---|---|---|---|
| VI | $\delta$ 5·78 | 1·22 | 1·49 |
| VII | 5·66 | 1·15 | 1·67 |
| VIII | 5·63 | 1·17 | 1·66 |

Subsequently, the $C_{11}$—$CH_3$ resonance appears at a field too high to assign the Me protons attached to acetoxyl-bearing C atoms. This should be attributed to the anisotropic shielding effects of the CO function[7] at $C_{10}$ and(or) the $\Delta^{4a,9a}$ double bond.[8] If $R_3$ in Fig. 4 represents the Me group, the explanation of the upfield-shift of the Me resonance seems impossible. In this case, a considerable downfield-shift due to the anisotropic effect[9] of the 1,3-diaxial like $C_3$—OH group is in keeping with the position of $R_3$ far from the above-mentioned shielding functions.




IX R = H
X R = $CH_3$

CHART 3

† The free rotation about $C_4$—$C_{11}$ linking seems to be difficult in view of inspection of molecular models.

The conformation of the $C_{11}$—OAc group was inferred from the intramolecular hydrogen-bonding of the $C_3$—OH. As I is insoluble in carbon tetrachloride, it was converted into the monoanhydro B-II (IX) and its trimethyl ether (X). The NMR spectrum of IX clearly shows that dehydration and aromatization of one unit had occurred. The IR spectra* of IX and X in dilute carbon tetrachloride (20 mm cell) are shown in Fig. 5. The broad band of IX in the low frequency region (3200–2900


FIG. 5 IR spectra of IX (1) and X (2) in dilute $CCl_4$.

$cm^{-1}$) must be due to the peri-OH groups because this band disappears on methylation. On the other hand, the two bands at 3611 $cm^{-1}$ and 3485 $cm^{-1}$ observed in both spectra must be attributed to $C_3$—OH stretching, and the pattern clearly indicates the existence of an equilibrium between free and hydrogen-bonded conformers about $C_3$—OH. Moreover, the large $\Delta\nu$ value (free $\nu_{OH}$ – bonded $\nu_{OH}$) suggests that the OH group must be bonded to the CO function,[10] which cannot be other than the $C_{11}$—OAc. These results together with inspection of the Dreiding models, confirm that the acetoxyl group is not situated at $R_2$ in Fig. 4.

The above spectroscopic evidence suggests one of the two possible conformers, A [$R_1$=OAc, $R_2$=$CH_3$, $R_3$=H] and B [$R_1$=$CH_3$, $R_2$=H, $R_3$=OAc], which are rotation isomers having the same configuration at $C_{11}$. Conformation B is unlikely because the strong interaction between the $C_{11}$—$CH_3$ group and the $C_2$-axial H atom would require a considerable decrease in the dihedral angle between $C_4$—H and $C_{11}$—H. Conformation A is supported by the upfield-shift (0·12–0·15 ppm) of $C_{11}$—H resonance on acetylation of the $C_3$—OH group (cf. Table 1). This fact clearly indicates that spatially, $C_{11}$—H is close to $C_3$—OH.

With regard to the quinone ring, one of two conformations is possible. In one of the two CO groups ($C_9$ and $C_{10}$) are upward orientated and in the other the orienta-

* The IR spectra were measured on a JASCO-DS402G (Grating) spectrometer for hydroxyl region.

tion is downward. As described above, the α-axial orientation of the $C_4$-sidechain must be due to release of its non-bonded interaction with the quinone carbonyl. Therefore, the upward orientation of the CO groups is favoured because of the more spatial arrangement as shown in Fig. 6. The spectroscopic evidence concerning the $C_{11}$-substituents are in accord with this conformation.

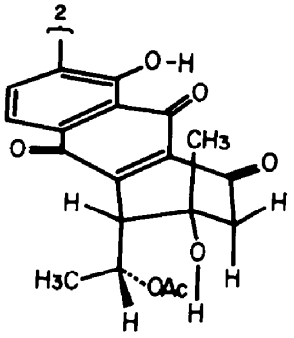



FIG. 6

In order to study the stereochemistry of the degradation products (IV and V), the NMR spectra of IV (in $CD_3COCD_3$) and V (in $CDCl_3$) were examined. In the spectra, the $C_4$—OH and $C_{3a}$—H protons exhibit broad signals. Since the $C_{3a}$—H signal was sharpened by the deuterium exchange experiment, there should be a long-range spin coupling (according to the W-letter arrangement) between $C_4$—OH and $C_{3a}$—H protons. Therefore, $C_4$—OH and $C_{3a}$—H must be *trans* diaxial. This evidence is compatible with the conclusion concerning the configuration of I at $C_3$ and $C_4$. In addition, it also suggests that the conformation of the hydroaromatic ring has been transformed during the degradation reactions. The transformation should originate in the disappearance of steric hindrance by the fission of the quinone ring and in lactone formation. Thus, the stereochemistry of IV and V at $C_4$ and $C_{3a}$ should be as illustrated in IVa and Va in Chart 4.

Treatment of Va with hydrogen bromide gave the bromohydrin (XI) in almost quantitative yield, and catalytic reduction of Va afforded the diol (XII). The structure of XII was confirmed by its NMR spectrum which shows a new tertiary Me signal, and by formation of the bismethoxycarbonate (XIII). On treatment with phosgen–pyridine complex, XI and XII gave the cyclic carbonates XIV and XV respectively. Thus, the configuration and conformation of these derivatives at C-6 is established as shown in Chart 4.

In relation to the configuration of $C_{11}$—H in I, the degraded compounds should have an α-hydrogen at $C_3$. Since the α-position is placed in nearly 1,3-diaxial relationship to the $C_4$—OH function, the anisotropic effect of the OH function to the $C_3$—H resonance can be predicted. Table 2 shows the chemical shifts of the $C_3$—H and $C_3$—$CH_3$ in $CDCl_3$. As expected, on acylation of the $C_4$—OH function the $C_3$—$CH_3$ signal does not show any upfield-shift, while the $C_3$—H resonance shows a slight upfield-shift. This observation[11] is in good accordance with the configuration at $C_3$ shown in Chart 4.

More positive proof regarding the anisotropic effect of the $C_4$—OH function was

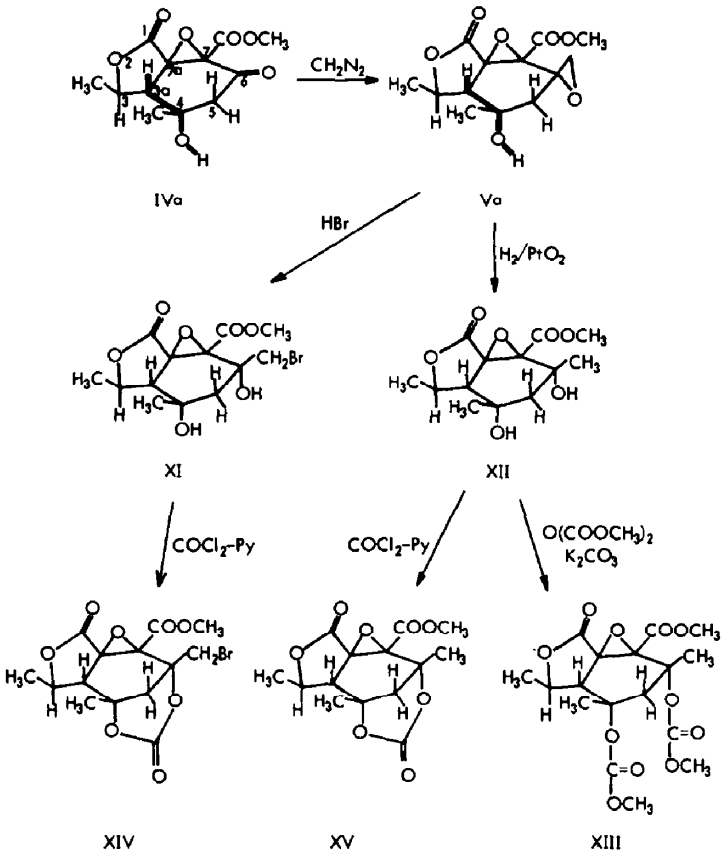



CHART 4

obtained from the NMR spectrum of XVI which was formed in addition to Va on treatment of IVa with diazomethane. Compound XVI has the molecular formula, $C_{14}H_{18}O_7$, which suggests the introduction of two $CH_2$ units into IVa, but on further treatment of Va with diazomethane, unchanged material was recovered. Compound XVI shows the presence of the γ-lactone (1795 $cm^{-1}$) and the α,β-epoxy ester (1760 $cm^{-1}$) but has neither OH nor ketone groups (in IR and ORD). The NMR spectrum (in $CDCl_3$) of XVI clearly exhibits an additional MeO signal (3·47

TABLE 2

| Compound | $C_3$--H | $C_3$—$CH_3$ |
|---|---|---|
| Va | δ 5·12 | 1·60 |
| XI | 5·06 | 1·62 |
| XII | 5·05 | 1·60 |
| XIII | 5·02 | 1·60 |
| XV | 5·00 | 1·63 |

ppm, 3H) other than the signal assignable to the COOMe group (3·78 ppm, 3H) and AA'BB'-type proton signals centered at 1·93 ppm, but the signals of $CH_2$ on the epoxide ring and the —C—$CH_2$—C— are absent. Therefore, the two $CH_2$ units must be introduced as O—$\underline{CH_3}$ and —C—$\underline{CH_2}$—$CH_2$—C— groups, respectively and not as a spiro epoxide. In fact, XVI does not give a bromohydrin or hydrogenated products. All this evidence together with a reasonable reaction pathway—ring expansion, conversion into hemiketal and further methylation of the hemiketal,[12] clearly indicates structure XVI as shown in Chart 5.

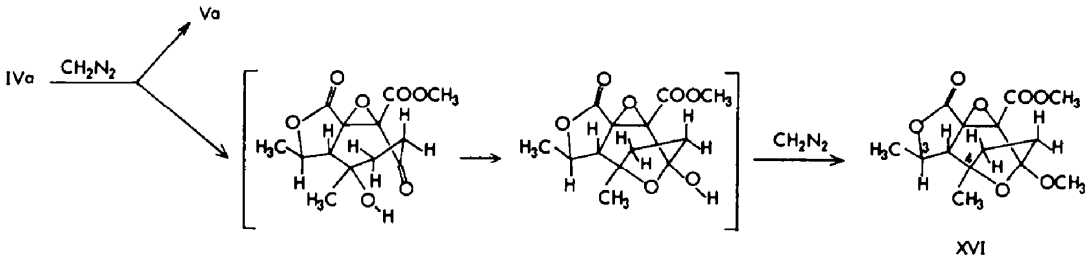



CHART 5

In the NMR spectrum of XVI the signals of $C_3$—H and $C_3$—$CH_3$ appear at 4·62 ppm (1H, doublets of quartet, $J$ = 6·0, $J$ = 10·0 c/s) and 1·60 ppm (3H, doublet, $J$ = 6·0 c/s), respectively. The $C_3$—H resonance, compared with the corresponding signals of Va, XI and XII (cf. Table 2), shows a considerable upfield-shift (0·4–0·5 ppm), but the $C_3$—$CH_3$ resonance remains unchanged. Since the upfield-shift can be attributed to the disappearance of the anisotropic effect of the $C_4$—OH group which has been situated close to $C_3$—H, this result supports the α-configuration of $C_3$—H.

In order to determine the configuration of the 7,7a-epoxide ring introduced in the course of oxidation, the intramolecular hydrogen bonding of XII was examined. Since XII as well as IVa and Va is insoluble in carbon tetrachloride, the IR spectra were measured* in chloroform at a concentration less than 0·005 mole per litre. The free tertiary OH stretching in chloroform was estimated by the OH band (3597 $cm^{-1}$) of t-butanol under the same conditions. The results obtained in a 5 mm cell† are shown in Fig. 7.

The spectrum of XII shows the presence of a strongly hydrogen-bonded OH band at 3427 $cm^{-1}$ and a very weak band at 3589 $cm^{-1}$ which is assigned as a free OH band. This pattern suggests that in addition to the mutual bonding between $C_4$—OH and $C_6$—OH, intramolecular hydrogen-bonding probably exists between the $C_6$—OH and the ester group at $C_7$. As shown in Fig. 7, the spectra of IVa and Va which lack the $C_6$—OH function in the molecule only exhibit free OH bands.

* See footnote* on page 7

† The spectrum of XII was also measured in a 1 mm cell and a 0·25 mm cell at varied concentrations. The absorption pattern showed no change by the concentration.

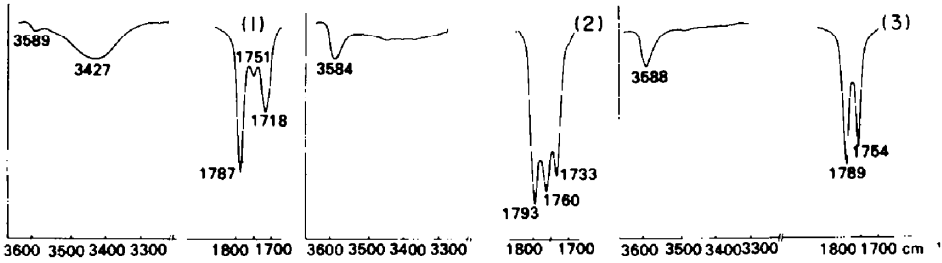



FIG. 7 IR spectra of XII (1), IVa (2) and Va (3) in dilute $CHCl_3$.

This conclusion agrees with the observation in the ester region. The ester bands of IVa and Va in the high frequency region (1750–1760 $cm^{-1}$), whether in the solid state or in solution, indicate that in these compounds the C=O dipole of the ester group is oriented in the same direction as the $C_7$—O.[3] As the ester band of XII at 1751 $cm^{-1}$ (in solid state) splits into the 1751 $cm^{-1}$ and 1718 $cm^{-1}$ bands in chloroform, there is probably an equilibrium between the conformer XIIa (of above-mentioned type) and conformer XIIb in which the dipoles are oriented in opposite directions as shown in Fig. 8. The intensity of both bands indicates the favoured conformer XIIb in solution contrary to the exclusive predominance of conformer XIIa in the solid state,* and the weak intensity at 1751 $cm^{-1}$ is proportional to the

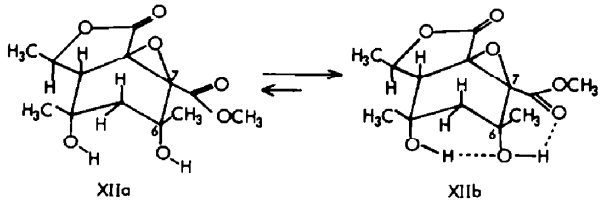



FIG. 8

very weak free OH intensity. The distinctive behaviour of XII in the solution could result from the stabilization of conformer XIIb by the intramolecular hydrogen-bonding between $C_6$—OH and the carbonyl O atom of the $C_7$-ester group, as supported by the considerably low frequency shift of the ester band. Since the formation of this intramolecular hydrogen-bonding is possible only in the configuration in which the ester group is *cis* to the α-quasiaxial $C_6$—OH function, the configuration of the 7,7a-epoxide ring must be β.

Last of all, the absolute configuration of I was conjectured by application of the empirical rules to the degradation products.

(1) *The application of the α,β-epoxy ketone rule*[13] *to* IVa. The ORD and CD curves† of IVa are shown in Fig. 9. The Cotton effects at 232 mμ and 303 mμ are due to a γ-lactone and an epoxy ketone, respectively. According to the rule, the positive

* Even in solid state, the free OH band was not observed presumably due to intermolecular hydrogen-bonding.

† ORD and CD curves were measured on a JASCO ORD/UV-5 attached with CD. $[\alpha]_D$ values were measured on a Perkin–Elmer polarimeter 141.

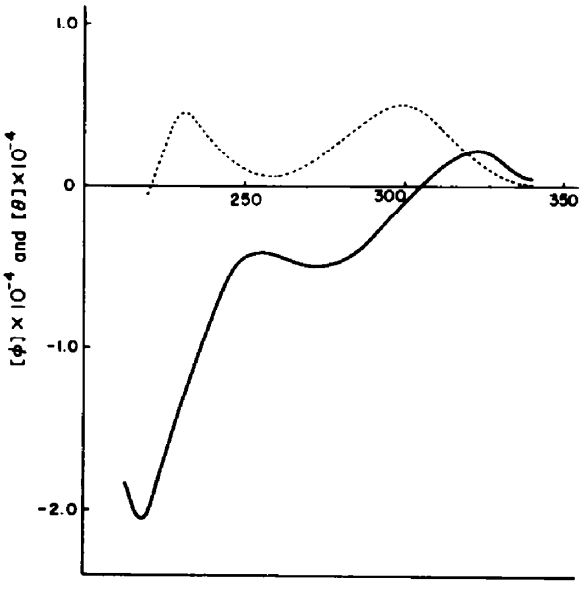



FIG. 9 ORD (——) and CD (- - -) curves of IVa in MeOH. Wavelength in mμ.

Cotton effect at 303 mμ indicates that the absolute configuration of the epoxide ring is β.

(2) *The application of Hudson's lactone rule*[14] *to* Va. The molecular rotation values for IVa and the corresponding potassium salt* are −97° and −196°, respectively. The positive $\Delta[M]_D$ value for the lactone minus the potassium salt suggests that the Me group on the lactone ring has the β-configuration.

Both results support the conclusion that the absolute configuration of I is probably as shown in Fig. 6 although some questions still remain: It is not yet clear whether the α,β-epoxyketone rule should be applied to a system which has a γ-lactone conjugated to an epoxide, and whether the lactone rule should be applied to a system having an epoxide conjugated to a γ-lactone, but the X-ray analysis of XI which is in progress should confirm the absolute configuration.

## EXPERIMENTAL†

*4,4'Diethyl*,1,1',8,8'-*tetrahydroxy*-3,3'-*dimethyl*-7,7'-*bianthraquinonyl* (III)

A suspension of 100 mg II in 20 ml MeOH was hydrogenated over 160 mg 40% Pd–C in the presence of 0·5 ml 10% NaOH. After uptake of 18·6 ml $H_2$ at 24°, the dark reddish purple soln turned to yellowish brown. The catalyst was filtered off and washed with MeOH. The combined soln which became soon dark red in the air was evaporated *in vacuo*. The residue was acidified with dil HCl and extracted with $CHCl_3$ to give 70 mg crude product, which was chromatographed on metal free silica gel[2] with $CHCl_3$. The eluate was evaporated and the residue was recrystallized from $CHCl_3$ to give 34 mg III as yellow needles, m.p. >290°. (Found: C, 72·47; H, 4·59. $C_{34}H_{26}O_8$ requires: C, 72·59; H, 4·66%). This product showed no acetoxyl band in IR spectrum and no optical activity in ORD.

* This value was obtained without isolation of K-salt. The cleavage of the lactone ring was confirmed by ORD.

† All m.ps are uncorrected.

*Oxidative degradation of* I *with* $H_2O_2$

Under stirring, 20 ml 5% NaOH was added to a soln of 1·0 g I in 30 ml dioxan and 16 ml 30% $H_2O_2$ without cooling. After 15 min, the soln was acidified with 10% HCl, and the excess $H_2O_2$ was decomposed with $PtO_2$. The mixture was filtered, and the filtrate was evaporated *in vacuo* to give dark brown residue, which was dissolved in water and washed with EtOAc. The water layer was adjusted to pH 5 with alkali and eluted on Dowex 50 (× 12 proton type) with water. The eluate was evaporated *in vacuo* to give about 950 mg acid mixture as a syrup.

(1) *Methylation with* MeI-$Ag_2O$ (*keto-lactone* IV). The above crude acid was methylated with excess MeI and anhyd $Ag_2O$ by refluxing in MeOH for 3 hr. The crude oily ester (1·01 g) was fractionated on 60 g silica gel with $CHCl_3$–MeOH (100:0·5–100:2). After elution of an oily mixture, 240 mg of a crude crystalline product was obtained. Recrystallization from $CHCl_3$ gave 103 mg pure IV as colourless prisms, m.p. 209–210°. (Found: C, 53·31; H, 5·22; OMe, 11·27; M.W. (osmometry) 285. $C_{12}H_{14}O_7$ requires: C, 53·33; H, 5·22; OMe, 11·48%; M.W., 270·23); UV $\lambda_{max}^{MeOH}$ mμ (ε): 207 (1300), 300 (37). $[\alpha]_D^{23}$ −19·9° ±2° (*c*, 0·855 MeOH); ORD: $[\phi]_{323}^{peak}$ +2270, $[\phi]_{275}^{trough}$ −5640, $[\phi]_{251}^{peak}$ −4750, $[\phi]_{218}^{trough}$ −21,100 (*c*, 0·0393 MeOH), CD: $[\theta]_{303}^{max}$ +4030, $[\theta]_{260}^{min}$ +511, $[\theta]_{232}^{max}$ +2900.

(2) *Methylation with diazomethane* (*epoxy-lactone* V). The crude acid mixture (obtained from 1·0 g I) was dissolved in THF and treated overnight with a large excess of ethereal diazomethane. The solvents were distilled off, and the residue was chromatographed on 60 g silica gel and eluted with $CHCl_3$–MeOH (100:0·5) to give total 196 mg crude crystalline product. It was purified by preparative TLC,* rechromatography on silica gel and finally by recrystallization from $CHCl_3$–ether to afford 44 mg colourless fine needles, m.p. 166–167°. (Found: C, 54·72; H, 5·61; OMe, 10·61; M.W., 287. $C_{13}H_{16}O_7$ requires: C, 54·93; H, 5·67; OMe, 10·92%; M.W., 284·26); $[\alpha]_D^{23}$ −34·2° ± 2° (*c*, 1·048 MeOH), $[\alpha]_D^{23}$ −57·6° ± 2° (after the addition of one drop MeOH–KOH); ORD: $[\phi]_{245}^{peak}$ +5200, $[\phi]_{205}$ −35,000; CD: $[\theta]_{225}^{max}$ +17,100 (*c*, 0·0491 MeOH).

*The treatment of* IV *with diazomethane* (V *and* XVI)

A soln of 100 mg IV in 5 ml MeOH was treated overnight with excess etheral diazomethane. Removal of the solvents and recrystallization of the residue from $CHCl_3$–ether gave 77 mg V. The preparative TLC of the mother liquor gave 10 mg additional pure crop (from bottom zone), total yield of V was 87 mg (83%).

From the upper zone of the preparative TLC, 15 mg (13·5%) XVI was isolated in almost pure state. Recrystallization from ether gave colourless fine plates, m.p. 177·5–178°. (Found: C, 56·37; H, 6·08. $C_{14}H_{18}O_7$ requires: C, 56·03; H, 6·02%); IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 1795 (γ-lactone), 1760 (α,β-epoxyester).

*The dehydration of* I *to monoanhydro* B-II (IX)

A soln of 55 mg I in 2 ml pyridine was allowed to stand at room temp for 6 hr. The soln was acidified with 10 ml 10% HCl under cooling. The ppts were extracted with EtOAc, and the EtOAc layer was thoroughly washed with water, dried over $MgSO_4$ and evaporated to give 48 mg amorphous powder. Preparative TLC on metal free silica gel† gave 5 mg bisanhydro B-II (II), 22 mg IX and 22 mg unchanged I. The compound IX was further purified from ether–n-hexane to give orange amorphous powder. (Found: C, 65·46; H, 4·62. $C_{38}H_{32}O_{13}$ requires: C, 65·51; H, 4·63%).

*The methylation of* IX *to* X

To a soln of 9 mg IX in 3 ml acetone were added 2 ml MeI and 20 mg anhyd $Ag_2O$, and the mixture was refluxed with stirring for 3 hr. The reaction mixture was filtered, and the evaporation of the filtrate gave a yellow product, which was purified by preparative TLC to afford 10 mg X. Further purification from EtOAc–n-hexane gave yellowish amorphous powder. (Found: C, 66·29; H, 5·40; OMe, 12·58. $C_{41}H_{38}O_{13}$ requires: C, 66·66; H, 5·19; OMe, 12·60%).

*Bromohydrin* (XI)

To a soln of 57 mg V in 5 ml $CHCl_3$, 3 ml $CHCl_3$ saturated with HBr was added and the mixture allowed to stand for 1 hr. After evaporation of the solvent *in vacuo*, recrystallization of the residue from $CHCl_3$–n-

* The PTLC of colourless compounds was carried out on Kiesel gel G (Merck) using $CHCl_3$—MeOH (9:1) system. For visualization iodine vapour was used.

† Yamani-layer PG (Yamani Chemical Co. Ltd., Osaka, Japan) was used without any treatment.

hexane gave 66 mg (90·5%) XI as colourless prisms, m.p. 198–199°. (Found: C, 42·54; H, 4·76; Br, 22·15. $C_{13}H_{17}O_7Br$ requires: C, 42·75; H, 4·69; Br, 21·88%); IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3315 (OH, broad), 1788 (γ-lactone), 1743 (COOMe).

*The catalytic hydrogenation of* V *to diol* XII

A soln of 45 mg V in 10 ml EtOH was hydrogenated over 20 mg $PtO_2$ for 3 hr. This hydrogenation gave two products; a $CHCl_3$ soluble product (32 mg) and a water soluble one (12 mg). Recrystallization of the former from EtOAc afforded pure XII as colourless prisms, m.p. 218–219·5° (Found: C, 54·39; H, 6·51; OMe, 10·60. $C_{13}H_{18}O_7$ requires: C, 54·54; H, 6·34; OMe, 10·84%); IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3290 (OH, broad), 1787 (γ-lactone), 1751 (COOMe).

The water soluble product (corresponding acid) was difficult to purify, but by methylation with MeI—$Ag_2O$ it gave XII.

*Bismethoxycarbonate of* XII (XIII)

A mixture of 4 mg XII, 1 ml dimethyl pyrocarbonate, and about 20 mg anhyd $K_2CO_3$ (finely powdered) was heated at 105–110° for 2 hr. The mixture was filtered and washed with $CHCl_3$. The combined soln was evaporated *in vacuo* to give 8 mg crude crystalline product. Recrystallization from EtOH gave 2 mg pure XIII as colourless prisms, m.p. 159–160° (on hot stage). (Found: C, 50·61; H, 5·59; OMe, 22·82. $C_{17}H_{22}O_{11}$ requires: C, 50·74; H, 5·51; OMe, 23·14%).

This compound was also obtained from above-mentioned acid; the acid (11 mg) was treated with dimethyl pyrocarbonate (3 ml) in the presence of $K_2CO_3$ for 4 hr as above. Recrystallization of the product from EtOH afforded 14 mg XIII, which was identical with the specimen from XII.

*Bromohydrin carbonate* (XIV)

To a soln of 14 mg XI in 5 ml $CHCl_3$ was added a soln of one ml pyridine and 0·5 ml liquid phosgen in 2 ml chloroform, and the soln was refluxed with stirring for 2 hr. The excess phosgen–pyridine complex was decomposed with $H_2O$ below 10°, and the mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with dil HCl, dried over $MgSO_4$ and evaporated to give 20 mg oily product, which was purified by preparative TLC. The pale yellow crystals (12 mg) were recrystallized from ether to afford 7 mg pure XIV as colourless plates, m.p. 195–197° (on hot stage). (Found: C, 43·21; H, 3·92; Br, 20·26; OMe, 7·99. $C_{14}H_{15}O_8Br$ requires: C, 42·98; H, 3·87; Br, 20·43; OMe, 7·93%); IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 1792 (carbonate), 1760 (γ-lactone), 1744 (ester). No absorption band was observed in the OH region.

*Diol carbonate* (XV)

In the similar method described for XIV, 32 mg XII was treated with a soln of 1·5 ml pyridine and 0·75 ml liquid phosgen in 7 ml $CHCl_3$. The crude product was purified by preparative TLC. Recrystallization of the main fraction from ether and then from MeOH gave 22 mg pure XV as colourless prisms, m.p. 175–176° (on hot stage). (Found: C, 53·73; H, 5·21; OMe, 9·84. $C_{14}H_{16}O_8$ requires: C, 53·84; H, 5·16; OMe, 9·94%); IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 1790 (carbonate), 1763 (γ-lactone) 1742 (ester), and no absorption band in OH region.

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## REFERENCES

[1] N. Tsuji, *Tetrahedron* **24**, 1765 (1968).
[2] J. Shōji, Y. Kimura and K. Katagiri, *J. Antibiotics Ser. A* **17**, 156 (1964).
[3] H. O. House and J. W. Blaker, *J. Am. Chem. Soc.* **80**, 6389 (1953).
[4] H. D. Dakin, *Am. Chem. J.* **42**, 477 (1909).
[5] S. Sternhell, *Rev. Pure Appl. Chem.* **14**, 15 (1964); A. Rassat, C. M. Jefford, J. M. Lehn and B. Waegell, *Tetrahedron Letters* 233 (1964).
[6] M. Karplus, *J. Chem. Phys.* **30**, 11 (1959); *J. Am. Chem. Soc.* **85**, 2870 (1963); N. S. Bhacca and D. H. Williams, *Applications of NMR Spectroscopy in Organic Chemistry* Chap 6. Holden-Day, San Francisco (1964).

[7] L. M. Jackman, *Applications of NMR Spectroscopy in Organic Chemistry* p. 122. Pergamon Press, London (1959).
[8] S. Yamaguchi, S. Okuda and N. Nakagawa, *Chem. Pharm. Bull.* **11**, 1465 (1963).
[9] R. F. Zürcher, *Helv. Chim. Acta* **46**, 2054 (1963).
[10] Y. Matsui, M. Takasuka and T. Kubota, *Ann. Rept. Shionogi Res. Lab.* **15**, 125 (1965).
[11] Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto and K. Tsuda, *Chem. Pharm. Bull.* **10**, 338 (1962); T. Okamoto and Y. Kawazoe, *Ibid.* **11**, 643 (1963).
[12] A. Gaudemer, J.-L. Fourrey and J. Polonsky, *Bull. Soc. Chim. Fr* **1967**(5), 1676.
[13] M. Legrand, R. Viennet and J. Caumartin, *C. R. Acad. Sci. Paris* **253**, 2378 (1961); C. Djerassi, W. Klyne, T. Norin, G. Ohloff and E. Klein, *Tetrahedron* **21**, 163 (1965).
[14] C. S. Hudson, *J. Am. Chem. Soc.* **61**, 1525 (1939).