Acyl Migration and Selective Esterification in Pyridoxol[1,2]

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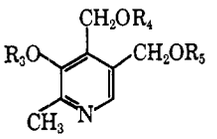
Acyl groups on the phenolic hydroxyl of pyridoxol have been found to migrate to the alcoholic hydroxyl in the 4 position. This rearrangement occurs with aromatic (benzoyl, *p*-nitrobenzoyl) and aliphatic (acetyl, palmitoyl) esters. The mechanism of this rearrangement has been studied. A rearrangement of this type takes place during partial esterification of pyridoxol with acid chlorides and explains the formation of aliphatic 3,4 diesters in high yields.

Since the first report of the preparation of a benzoyl derivative of pyridoxol by Birch and Gyorgy[3] in 1936, interest in the carboxylic acid esters of this vitamin has not abated, and such esters have been utilized in various phases of vitamin B_6 research.[4,5]

In the present study we have examined methods for the partial esterification of pyridoxol.[6]

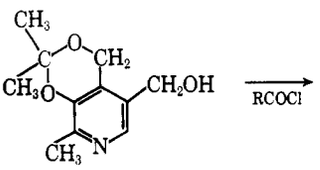


I

Mono-, di-, and trisubstituted esters of pyridoxol (I, $R_3 = R_4 = R_5 = H$) have been described (Table I). A fully esterified, trisubstituted compound is usually obtained by treating pyridoxol with an excess of an acid chloride in the presence of an acid acceptor.[3,7-9] It has recently been shown that aliphatic 3,α^4-O diesters of pyridoxol (I, $R_3 = R_4 = COR$; $R_5 = H$) can be obtained in excellent yield by the interaction of 2 moles of an acid chloride with 1 mole of pyridoxol in the presence of pyridine.[10] α^4,α^5-O diesters are also readily available. α^4,α^5-O-Diacetylpyridoxol has been obtained from pyridoxol by refluxing it with acetic acid,[11] or by interaction of the readily available 3,4-bis(bromomethyl)-5-hydroxy-6-methylpyridine with silver acetate,[12] the latter reaction exemplifying a method that could be generally applied to the synthesis of this type of esters.

Synthesis of pyridoxol monoesters requires special methods. So far, only α^5-O monoesters have been described,[8,13,14] although the preparation of an impure 3-O-palmitoylpyridoxol has been claimed in the literature.[8]

α^5-O esters (IV) have been obtained by the interaction of α^4,3-O-isopropylidenepyridoxol (II) with acid chlorides to yield the corresponding esters (III), followed by hydrolysis (eq 1) of the isopro-



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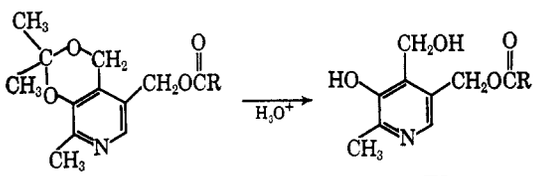
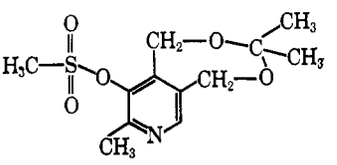


III IV (1)



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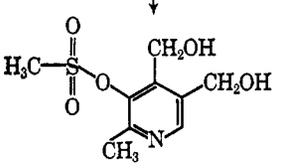

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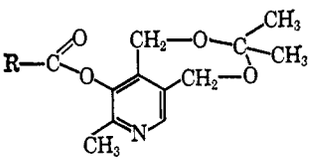
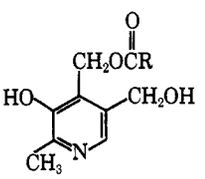


VI



VIII (3)

(1) Pyridoxine Chemistry. XV. Preceding papers in this series: W. Korytnyk, B. Paul, A. Bloch, and C. A. Nichol, *J. Med. Chem.*, **10**, 345 (1967); B. Paul and W. Kortynyk, *Chem. Ind.* (London), 230 (1967).

(2) Presented in part at the 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965, Abstracts, p 8P.

(3) T. W. Birch and P. György, *Biochem. J.*, **30**, 304 (1936).

(4) R. P. Singh and W. Korytnyk, *J. Med. Chem.*, **8**, 116 (1965), and others.

(5) W. Korytnyk, G. Fricke, and B. Paul, *Anal. Biochem.*, **17**, 66 (1966).

(6) Some aspects of the work have been reported briefly: W. Korytnyk and B. Paul, *Tetrahedron Letters*, No. 8, 777 (1966).

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(8) T. Sakuragi and F. A. Kummerow, *J. Am. Chem. Soc.*, **78**, 839 (1956).

(9) M. V. Balyakina, E. S. Zhdanovich, A. G. Zemskova, and N. A. Preobrazhenskii, *Zh. Obshch. Khim.*, **32**, 1172 (1962); *Chem. Abstr.*, **58**, 2429c (1963).

(10) (a) M. Uchibayashi, *Chem. Pharm. Bull* (Tokyo), **9**, 182 (1961). (b) Japanese Patent 6536 (1964); *Chem. Abstr.*, **61**, 13289[b] (1964). (c) British Patent 965,844 (1964); *Chem. Abstr.*, **61**, 11975[e] (1964).

(11) (a) S. A. Harris, D. Heyl, and K. Folkers, *J. Am. Chem. Soc.*, **66**, 2088 (1944). (b) French Patent M2514 (1964); *Chem. Abstr.*, **61**, 9471[d] (1964). (c) Belgian Patent 640,827 (1964); *Chem. Abstr.*, **63**, 587[h] (1965).

(12) S. A. Harris, *J. Am. Chem. Soc.*, **62**, 3203 (1940).

(13) J. Baddiley and A. P. Mathias, *J. Chem. Soc.*, 2583 (1952).

(14) W. Korytnyk and W. Wiedemann, *ibid.*, 2531 (1962).

TABLE I
ESTERS OF PYRIDOXOL

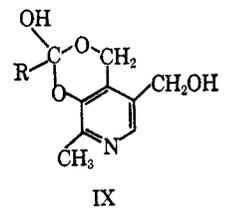
Degree of substitution	Type (structure no.)	Position (see structure I) R_3	R_4	R_5	References
Trisubstituted	Acetyl	$COCH_3$	$COCH_3$	$COCH_3$	*a*, *b*, ref 12, and this study
	Benzoyl	COPh	COPh	COPh	*c*
	Palmitoyl and other long-chain aliphatic acyls	$COC_{15}H_{31}$	$COC_{15}H_{31}$	$COC_{15}H_{31}$	Ref 8 and 9
Disubstituted	Acetyl	H	$COCH_3$	$COCH_3$	Ref 11a, ref 11b, ref 12
	Acetyl	$COCH_3$	$COCH_3$	H	Ref 10a
	Benzoyl	H	COPh	COPh	This study
	Benzoyl	COPh	COPh	H	This study
	Palmitoyl and other long-chain aliphatic acyls	$COC_{15}H_{31}$	$COC_{15}H_{31}$	H	Ref 10a
Monosubstituted	Acetyl (VIII)	H	$COCH_3$	H	This study
	Benzoyl (IV)	H	H	COPh	Ref 4, 14, 15
	Benzoyl (VIII)	H	COPh	H	This study
	p-Nitrobenzoyl (IV)	H	H	*p*-$COPhNO_2$	Ref 13 and 14
	p-Nitrobenzoyl (VIII)	H	*p*-$COPhNO_2$	H	This study

[a] R. Kuhn and G. Wendt, *Ber.*, **71**, 780 (1938). [b] W. Korytnyk, E. J. Kris, and R. P. Singh, *J. Org. Chem.*, **29**, 574 (1964). [c] T. W. Birch and P. György[3] were the first to obtain a benzoylpyridoxol that lacked vitamin activity. They did not provide further description of the properties of the substance obtained. A crystalline tribenzoate (mp 121–122°) was obtained by A. Ichiba and K. Michi; see ref 7.

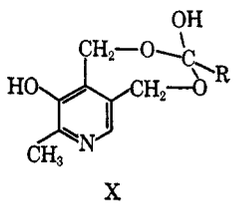
pylidene group.[8,13–15] We decided to apply the same method to the synthesis of 3-O esters, using the now available α^4,α^5-isopropylidenepyridoxol.[16] The phenolic hydroxyl could be readily esterified with methanesulfonyl chloride or benzoyl chloride, giving the corresponding esters V and VI. Hydrolysis of the isopropylidene group in V with acid yielded a methylsulfonylpyridoxol that gave a negative Gibbs test, indicating that the phenolic hydroxyl is substituted, as in VII (eq 2). Hydrolysis of the benzoate (VI, R = Ph), on the other hand, yielded a product that gave a positive Gibbs test (eq 3), indicating that the phenolic hydroxyl is unsubstituted, unless a rearrangement took place in the course of testing.

The following chemical evidence indicated that the rearranged product is a monobenzoyl derivative with a free phenolic group. Elemental analysis and infrared and nmr spectroscopy indicated that the product is a monobenzoylpyridoxol, but its melting point and other physical properties were different from those obtained for α^5-O-benzoylpyridoxol (IV, R = Ph). Interaction of the two isomeric monobenzoates with diazomethane[17] gave two different monomethyl ethers. Similarly, the phenolic hydroxyl in the benzoates could be preferentially reacted with methanesulfonyl chloride to yield two isomeric methanesulfonyl derivatives. Thus chemical evidence supports the α^4-O-benzoyl structure VIII.

Alternative structures considered are the *ortho* acids IX and X, which are ruled out by infrared spectroscopy. The possibility also exists that the 3-O-benzoyl derivative was the product, but that the rearrangement took place during the formation of derivatives, as well as during the Gibbs test. This possibility could not be ruled out by infrared spectroscopy alone, since the phenolic hydroxyl in pyridoxol derivatives is strongly hydrogen bonded, and does not show up as a discrete peak. An nmr study of these esters was undertaken in order to develop structural criteria for the benzoates. These studies were aided by the observation that in DMSO the proton exchange of the alcoholic hydroxyl is slowed down sufficiently to permit its resonance to be observed, provided the phenolic group is substituted, as in α^4,3-O-isopropylidenepyridoxol.[18,19] In this compound, the unsubstituted primary hydroxyl proton appears as a triplet and splits the adjacent methylene protons into a doublet. Addition of D_2O to the DMSO solution results in exchange, with collapse of the methylene doublet to a singlet.



IX



X

The presence of an unsubstituted phenolic hydroxyl in pyridoxol or its derivatives not only converts all of the hydroxyl protons to singlets (as has been observed for pyridoxol), but also broadens them in most cases, making them undetectable in the spectrum. Accordingly, no peaks corresponding to hydroxyl protons could be detected in α^5-O-benzoylpyridoxol and for the new benzoyl derivative, confirming its structure as being α^4-O-benzoylpyridoxol. As expected, blockage of the phenolic hydroxyl of the two isomeric benzoyl derivatives by a methyl group results in a normal appearance for the primary hydroxyl resonance (see Experimental Section).

The preceding chemical and physical evidence strongly supports formulation of the compound as α^4-O-benzoylpyridoxol. Hydrolysis of the isopropylidene group and rearrangement proceed under very mild conditions. Thus it has been established that a brief period of heating or merely keeping an aqueous solution of the hydrochloride of VI in water results in the formation of α^4-O-benzoylpyridoxol in good yield.

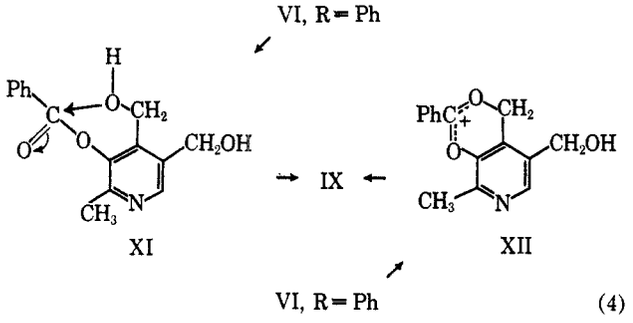
Analogous rearrangements were shown to occur in other aromatic and aliphatic esters, namely the 3-O-*p*-nitrobenzoyl, 3-O-palmitoyl, and 3-O-acetyl esters of

(15) U. Schmidt and G. Giesselmann, *Ann. Chem.*, **657**, 162 (1962).
(16) W. Korytnyk, *J. Org. Chem.*, **27**, 3724 (1962).
(17) D. A. Prins, *Rec. Trav. Chim.*, **76**, 58 (1957).
(18) W. Korytnyk and B. Paul, *J. Heterocyclic Chem.*, **2**, 481 (1965).
(19) O. L. Chapman and R. W. King, *J. Am. Chem. Soc.*, **86**, 1256 (1964).

α^4,α^5-isopropylidenepyridoxol. In addition to the α^4-O esters produced by rearrangement α^5-O esters have been obtained by independent methods of synthesis (as shown in II to IV), with the exception of α^5-O-acetylpyridoxol (Table I). A reasonable structural assignment for the α^4-O-acetyl derivative can be made by comparing the position of its methylene peaks with the positions of those of other α^4-O esters.

Most likely the rearrangement proceeds *via* the $3,\alpha^4$-*ortho*-acid intermediate IX, which is sterically favorable because of its six-membered *ortho*-acid ring. Since an acyl shift from α^4 to α^5 or vice versa has not been observed, it can be concluded that the formation of a cyclic *ortho* acid with a seven-membered ring including the α^4 and α^5 positions (X) is less favorable, although a cyclic ketal with a seven-membered ring including these positions is formed quite readily.[16]

Hydrolysis of the isopropylidene group may precede an attack on the ester carbonyl by the α^4-hydroxyl, as shown in XI. Alternatively, the isopropylidene group in VI might be displaced by an attack on the α^4-C atom in VI to form the carboxonium ion XII, which would then pick up a hydroxyl ion from water to yield the *ortho* acid (eq 4). When the rearrangement



(4)

reaction was carried out in O^{18}-enriched water, only a small fraction of the O^{18} (approximately 10%) was incorporated into the α^4 ester VIII.[20] Thus formation of the carboxonium ion intermediate XII can be ruled out as a major pathway, and the rearrangement most likely proceeds in two steps, namely the initial hydrolysis of the isopropylidene group, yielding the 3-O-benzoyl derivative XI, and then rearrangement.

The selectivity of the esterification of polyfunctional compounds is sometimes dependent on acyl rearrangements.[21] The great ease with which acyl migration takes place in esters of pyridoxol led us to investigate the relationship of the migration to the selective esterification reactions of the compound. Uchibayashi[10a] noted that the 3- and α^4-hydroxy groups of pyridoxol show a remarkably high selectivity in esterification with aliphatic acid chlorides, a reaction that has been applied to the synthesis of a number of $3,\alpha^4$-O diesters of pyridoxol. The explanation put forward for this selectivity was the acidity of the phenolic hydroxyl and the activation of the α^4-hydroxyl by the pyridine ring.[10a] We believe that the latter effect is too small for the pronounced selectivity observed, and offer an alternative explanation based on the following considerations.

Monoacylation of the two isomeric benzoylpyridoxols (IV and VIII, R = Ph) and of α^4-O-*p*-nitrobenzoylpyridoxol (VIII, R = *p*-$PhNO_2$) can be readily achieved with methanesulfonyl chloride in pyridine, yielding 3-O-substituted derivatives. Benzoyl chloride, on the other hand, gives different results. Monoesterification of α^5-O-benzoylpyridoxol with 1 mole of benzoyl chloride in pyridine gave α^4,α^5-O-dibenzoylpyridoxol. If the 4 position was substituted, as in α^4-O-benzoylpyridoxol, the expected $3,\alpha^4$-O-dibenzoylpyridoxol was obtained. Although it is almost certain that the phenolic hydroxyl is esterified first in both cases, the 3-O-benzoyl in the $3,\alpha^5$-O-disubstituted compound initially formed from the α^5-O-monosubstituted benzoate has the opportunity to migrate to the 4 position, thus giving the α^4,α^5-O isomer, whereas the α^4-O position in the α^4-O-monosubstituted compound is already occupied, and hence the expected $3,\alpha^4$-O-dibenzoyl derivative is obtained. The selectivity of the diesterification of pyridoxol, leading to $3,\alpha^4$-O diesters,[10] can be explained by a three-stage mechanism. Monoesterification of the phenolic hydroxyl is followed by an immediate shift of the acyl group to the 4 position. The liberated phenolic hydroxyl is then subject to another esterification, this time not giving a rearranged product. The rate of the rearrangement step can be expected to be greater than that of the esterification step, provided that the selectivity is maintained.

Although aliphatic acid chlorides have been extensively studied in connection with the selectivity of the esterification of pyridoxol, aromatic acid chlorides have not as yet been utilized to any appreciable extent in the direct synthesis of partially esterified pyridoxols. We have subjected pyridoxol hydrochloride to "monobenzoylation," using a 20% excess of benzoyl chloride in a mixture of pyridine and chloroform. In addition to α^4-O-benzoylpyridoxol, which was the predominant product, we also obtained $3,\alpha^4$-O- and α^4,α^5-O-dibenzoylpyridoxol. The presence of the second of the latter products indicates that the selectivity is not so great for benzoylation as it is for acylation with the aliphatic acid chlorides used thus far. The reason for this lesser selectivity may be related to the greater stability of the intermediate *ortho* acid IX derived from the 3-O-benzoylpyridoxol initially formed, as compared with the corresponding aliphatic intermediates, which apparently exist a much shorter time. No 3-O- or α^5-O-monobenzoylpyridoxol could be detected among the reaction products, thus lending support to the proposed sequence of reactions.

Experimental Section

Thin Layer Chromatography (Tlc).—The plates were coated with 250 μ of silica gel "G," were dried at room temperature overnight, and were heated at 100° for 30 min. They were developed with organic solvents (ethyl acetate, chloroform, or methanol, or mixtures of those solvents), sprayed with the Gibbs reagent (1% dichloroquinone chlorimide in benzene or toluene), and held over the open mouth of a bottle of NH_4OH solution or sprayed with 1 *N* NaOH. Development of blue spots indicates that the phenolic group is unsubstituted. Pyridoxol derivatives that have the phenolic group blocked are indicated by spraying with either 1 *N* HCl or 1 *N* NaOH, depending on whether the blockage is acid hydrolyzable (*e.g.*, methoxy substitution) or base hydrolyz-

(20) We thank Dr. D. C. DeJongh, Chemistry Department, Wayne State University, for recording the mass spectrum and assisting in its interpretation. The spectrum was obtained with an Atlas CH 4 mass spectrometer at an ionizing potential of 70 ev and an ionizing current of 18 ma, using a direct inlet introduction system.

(21) A. C. Richardson and J. M. Williams, *Chem. Comm.*, 104 (1965).

able (*e.g.*, methanesulfonyl or other acyl substitution), and subsequently heating at *ca.* 100° till the plate appears to be dry. The plate is then treated with the Gibbs reagent, and development is continued in the manner already described.

Infrared spectra were determined with a Perkin-Elmer 137B spectrophotometer.

Nmr spectra were determined in a Varian A-60 instrument. The solvent was dimethyl sulfoxide in which 10–15% of the compound was dissolved; positions of peaks are expressed in cycles per second from tetramethylsilane as an internal standard. The instrument was calibrated by the method of Jungnickel,[22] and the positions of peaks are accurate within 1 cps. Assignments of peaks were made on the basis of previous work.[18] Methylene peak assignments was made possible by comparison with the spectra of their monomethyl ethers, in which the methylene peaks adjacent to the unsubstituted hydroxyls can be identified readily. It was found that methylation did not drastically change the positions of the methylene peaks in the parent compound, and thus the assignment of peaks appears to be correct beyond reasonable doubt. This assignment is also supported by the extent of the shift (approximately −50 cps) of the methylene protons on benzoylation.

3-O-Benzoyl-α^4,α^5-O-isopropylidenepyridoxol (VI, R = Ph) was prepared as previously described:[15] yield 88%, mp 102–104° (lit.[16] mp 107–109°).

An attempt to apply the Schotten–Baumann reaction (α^4,α^5-O-isopropylidenepyridoxol and benzoyl chloride in the presence of 10% NaOH) gave less satisfactory results. The benzoate was obtained in poor yield and was contaminated with a product ("X") that had an unsubstituted phenolic group. Tlc was carried out with a 4:1 mixture of ethyl acetate and chloroform: α^4,α^5-O-isopropylidenepyridoxol, R_f 0.16; compound "X", R_f 0.36; 3-O-benzoate, R_f 0.64.

α^4-O-Benzoylpyridoxol (VIII, R = Ph).—3-O-Benzoyl-α^4,α^5-isopropylidenepyridoxol (5.00 g, 16 mmoles) was suspended in 10% formic acid (400 ml) and was heated on a steam bath for 20 min. After evaporation *in vacuo*, alcohol was added to the residue, and the material was evaporated again. (Tlc with 9:1 $CHCl_3$–MeOH at this stage produced at least seven spots.) The residue crystallized, yielding 2.36 g (7.5 mmoles) of crystals, mp 134–136°. Besides the main spot at R_f 0.35, there were several weak spots, especially at R_f 0.0 and 0.69. After crystallization several times from ethanol and twice from water, pure material was obtained: mp 141–142°; λ_{max}^{Nujol} 1704 (C=O), 3367 (alcoholic OH), 2688 (bonded OH), 717 cm^{-1} (benzoyl CH); nmr, 4-CH_2 −332 (s), 5-CH_2OH −281 (s), and COPh (m).

Anal. Calcd for $C_{15}H_{15}NO_4$: C, 65.89; H, 5.53; N, 5.13. Found: C, 65.62; H, 5.60; N, 4.99.

α^4-O-Benzoylpyridoxol (VIII, R = Ph) Hydrochloride.—3-O-Benzoyl-α^4,α^5-isopropylidenepyridoxol (200 mg) was dissolved in 1 *N* HCl (20 ml) and was heated on a steam bath. Aliquots were removed at various times, spotted on a tlc plate, and developed with a 1:1 methanol–chloroform mixture. Uniform positive spots were produced with the Gibbs reagent both initially and after heating for 15 min. A major portion of the solution from this experiment was evaporated *in vacuo*, water was added and evaporated again, and the product was crystallized from ethanol and recrystallized from an ethanol–ether mixture: mp 147°.

Anal. Calcd for $C_{15}H_{16}ClNO_4 \cdot 1.5H_2O$: C, 53.49; H, 5.69; N, 4.16. Found: C, 53.93; H, 5.67; N, 4.15.

3-O-Benzoyl-α^4,α^5-isopropylidenepyridoxol (VI, R = Ph) Hydrochloride.—Dry hydrogen chloride gas was bubbled into an ether solution of 3-O-benzoyl-α^4,α^5-isopropylidenepyridoxol. The isolated hydrochloride was recrystallized from an ethanol–ether mixture: mp 142–144°.

Anal. Calcd for $C_{18}H_{20}NO_4Cl$: C, 61.80; H, 5.70; N, 4.03. Found: C, 61.61; H, 5.81; N, 3.94.

Hydrolysis and Rearrangement.—The preceding hydrochloride was heated with water for 1 hr on a steam bath. Tlc with 50:50 MeOH–$CHCl_3$ indicated no starting material; the major spot was that of α^4-O-benzoylpyridoxol (R_f 0.87), and there was an unidentified minor spot (R_f 0.68). Hydrolysis and rearrangement took place in aqueous solution even on standing at room temperature for 24 hr.

Rearrangement of 3-O-Benzoyl-α^4,α^5-isopropylidenepyridoxol in O^{18}-Enriched Water.—3-O-Benzoyl-α^4,α^5-isopropylidenepyridoxol (10 mg) was dissolved in 1.0 ml of H_2O ((11.11% O^{18}-enriched and normalized to natural abundance of hydrogen isotopes; supplied by Volk Radiochemical Co.); 0.01 ml of concentrated hydrochloric acid was added; and the solution was heated for 30 min on a steam bath. The water was removed *in vacuo*, and the half-solid residue was found to be pure α^4-O-benzoylpyridoxol by tlc; it was subjected as such to mass spectrometry (Table II).

TABLE II

	Peak intensities		
	M	M + 1	M + 2
Hydrolysis product	100	17.7	2.9
Theoretical	100	17.0	1.5

3-O-Methyl-α^4-O-benzoylpyridoxol.—α^4-O-Benzoylpyridoxol (0.98 g, 3.18 mmoles), suspended in a mixture of *t*-butyl alcohol and ether, was cooled to −25° (Dry Ice). Diazomethane (*ca.* 30 mmoles) in ether was added dropwise for 3 hr. Then the solution was left standing overnight and was allowed to reach room temperature. After evaporation *in vacuo*, the resulting oily product was taken up in ethyl acetate, from which 0.57 g (55%) of crystalline material was obtained. Recrystallization from a mixture of ethyl acetate and ether and sublimation *in vacuo* raised the melting point to 100°; nmr, 4-CH_2 −330 (s), 5-CH_2OH −280 and −284 (d), 5-CH_2OH −319 (t), C_6-H −501, and COPh (m).

Anal. Calcd for $C_{16}H_{17}NO_4$: C, 66.88; H, 5.97; N, 4.68. Found: C, 66.95; H, 6.14; N, 4.77.

3-O-Methyl-α^5-O-benzoylpyridoxol.—α^5-O-Benzoylpyridoxol (2.2 g, 7.3 mmoles) reacted with diazomethane under the same conditions as were used for the 4-*O*-benzoyl isomer. The yield of pure material crystallized from ethyl acetate was 0.70 g, mp 138–140°. After HCl was passed in, the mother liquor yielded 0.70 g of a crystalline hydrochloride. The free base was analyzed: nmr, 4-CH_2OH −280 and −284 (d), 4-CH_2OH −317 (t), 5-CH_2 −334 (s), C_6-H −504 (s) and COPh (m).

Anal. Calcd for $C_{16}H_{17}NO_4$: C, 66.88; H, 5.97; N, 4.80. Found: C, 66.77; H, 5.78; N, 4.80.

3-O-Methanesulfonyl-α^5-O-benzoylpyridoxol.—α^5-O-Benzoylpyridoxol (1.90 g, 6.95 mmoles) was suspended in pyridine (20 ml), cooled with ice, and magnetically stirred. Methanesulfonyl chloride (0.70 g) in pyridine was added dropwise for 1 hr. After standing overnight at room temperature, water (150 ml) was added, and the solution was extracted with three 75-ml portions of ether. The compound was crystallized from ether and yielded 1.58 g (4.5 mmoles, 65% yield) of the mesylate, mp 133–134°. Recrystallization from ethanol raised the melting point to 138°.

Anal. Calcd for $C_{16}H_{17}NO_6S$: C, 54.64; H, 4.88; S, 8.70. Found: C, 54.44; H, 4.89; S, 8.96.

"Monobenzoylation" of α^5-O-Benzoylpyridoxol. α^4,α^5-O-Dibenzoylpyridoxol.—α^5-O-Benzoylpyridoxol (684 mg, 2.50 mmoles) in cold pyridine was let react with benzoyl chloride (0.347 ml, 3 mmoles) overnight. Water (50 ml) was added, and the solution was extracted with four 50-ml portions of chloroform. After evaporation of the chloroform extracts *in vacuo*, the residual oil was taken up in ethanol and evaporated, when crystallization took place. Recrystallization from ethanol gave needles (570 mg, 60%), mp 143–146°; another crystallization gave mp 143–144°. Tlc (1:9 methanol–chloroform) gave a single spot (R_f 0.76), which was Gibbs positive.

Anal. Calcd for $C_{22}H_{19}NO_5$: C, 70.02; H, 5.07; N, 3.71. Found: C, 70.28; H, 4.93; N, 3.79.

α^4,α^5-O-Dibenzoyl-3-O-methanesulfonylpyridoxol was obtained from the preceding dibenzoylpyridoxol by letting it react with methanesulfonyl chloride in pyridine for 3 days. The product was crystallized from alcohol, mp 93°.

Anal. Calcd for $C_{23}H_{21}NO_7S$: C, 60.65; H, 4.65; N, 3.08; S, 7.04. Found: C, 60.50; H, 4.79; N, 2.97; S, 6.87.

"Dibenzoylation" of α^5-O-Benzoylpyridoxol. Tri-O-benzoylpyridoxol.—α^5-O-Benzoylpyridoxol (683 mg, 2.50 mmoles) was dissolved in pyridine and cooled (0°), and benzoyl chloride (0.7 ml, 6 mmoles) was added. The solution was shaken for 4 hr. Water was added, and the solution was extracted with four 50-ml portions chloroform. After drying and evaporation *in vacuo*, an oily material was obtained. This was crystallized from absolute ethanol, giving 1.03 g, mp 123–126°, which was raised to 126° after another crystallization from ethanol (lit.[7] mp 121–122°).

Anal. Calcd for $C_{29}H_{22}NO_6$: C, 72.34; H, 4.81; N, 2.93. Found: C, 72.17; H, 4.87; N, 2.88.

"Monobenzoylation" of α^4-O-Benzoylpyridoxol. 3,α^4-O-Dibenzoylpyridoxol.—α^4-O-Benzoylpyridoxol (150 mg) was dis-

(22) J. L. Jungnickel, *Anal. Chem.*, **35**, 1985 (1963).

solved in 5 ml of dry pyridine and cooled in ice, and benzoyl chloride (0.062 ml) in 4 ml of anhydrous ether was added drop by drop with stirring over a period of 10 min. The reaction mixture was stirred in the cold for 1 hr and then at room temperature for 2 hr. The solvent was then evaporated completely *in vacuo*. The residue was cooled in ice, and crushed ice was added, when gummy material separated out. Tlc (ethyl acetate) of the gummy material showed one major spot (R_f 0.47) and two very minor spots (R_f 0.14 and 0.8 respectively). The reaction product was purified by preparative tlc with ethyl acetate, eluted with ether, and washed with 0.05 *N* NaOH solution and water. The ether extract was dried over anhydrous magnesium sulfate, filtered, and evaporated to a small volume. On treatment with an ethereal HCl solution, a white precipitate of the hydrochloride separated out. After filtration and washing with ether, it was crystallized from a methanol–ether mixture and yielded 204 mg (90%) of the hydrochloride: mp 152–154°; nmr, 4-CH_2 −330 cps (s), 5-CH_2OH −289 and −294 (d), 5-CH$_2$OH −338 (tr), and COPh (m).

Anal. Calcd for $C_{22}H_{20}NO_5Cl$: C, 63.84; H, 4.83; N, 3.38. Found: C, 64.01; H, 4.73; N, 3.54.

"Monobenzoylation" of Pyridoxol Hydrochloride. A.—Pyridoxol hydrochloride (2.06 g, 10 mmoles) was suspended in a mixture of pyridine and chloroform, benzoyl chloride (1.34 ml, 12 mmoles) was added, and the reaction mixture was shaken for 5 days. Chloroform (100 ml) was added, and the mixture was extracted with four 100-ml portions of water. After drying, the chloroform solution was evaporated, and the residue was examined by tlc (1:9 methanol–chloroform). Two spots were Gibbs positive before hydrolysis with sodium hydroxide: R_f 0.25 (α^4-O-benzoylpyridoxol) and R_f 0.70 (α^4,α^5-di-O-benzoylpyridoxol). A spot at R_f 0.53 was visible after hydrolysis, and was identical with that of 3,α^4-O-dibenzoylpyridoxol.

B.—Pyridoxol hydrochloride (2.06 g, 10 mmoles) was dissolved in dimethylformamide (50 ml) and pyridine (3.2 ml) by shaking and warming. Benzoyl chloride (0.72 ml, 6.2 mmoles) was added, and the solution was shaken overnight and then filtered. The residue consisted mainly of unreacted pyridoxol hydrochloride and some α^4-O-benzoylpyridoxol. The filtrate and residue were combined, water was added, and the mixture was extracted with chloroform, as in method A. The procedure yielded the same three products as the products obtained in method A.

3-O-*p*-Nitrobenzoyl-α^4,α^5-isopropylidenepyridoxol.—To a solution of α^4,α^5-isopropylidenepyridoxol (1.0 g, 4.8 mmoles) in pyridine, *p*-nitrobenzoyl chloride (7.2 mmoles) was added. After shaking for 30 min and the addition of water, the *p*-nitrobenzoate crystallized. Recrystallization from aqueous alcohol gave 1.44 g of pure compound, mp 164–166°.

Anal. Calcd for $C_{18}H_{18}N_2O_6$: C, 60.44; H, 5.03; N, 7.85. Found: C, 60.22; H, 5.15; N, 7.81.

α^4-O-*p*-Nitrobenzoylpyridoxyl Hydrochloride.—3-O-*p*-Nitrobenzoyl-α^4,α^5-isopropylidenepyridoxol (102 mg, 0.29 mmoles) was dissolved in 2.9 ml of 0.01 *N* HCl and 25 ml of 10% formic acid, and was heated for 45 min on a steam bath. After evaporation *in vacuo* and crystallization from absolute ethanol, 55.1 mg of the hydrochloride was obtained: mp 197°; nmr, 4-CH$_2$ −341 (s), 5-CH$_2$ −292 (s), *p*-COPhNO$_2$ −497 and −500 (d).

Anal. Calcd for $C_{15}H_{15}N_2O_6Cl$: C, 50.79; H, 4.26; N, 7.90. Found: C, 50.68; H, 4.47; N, 7.97.

3-O-Methanesulfonyl-α^4-O-*p*-nitrobenzoylpyridoxol.—α^4-O-*p*-Nitrobenzoylpyridoxol (248 mg, 0.70 mmole) was dissolved in pyridine, and the solution was cooled with ice. Methanesulfonyl chloride (0.066 ml), dissolved in cold pyridine, was added dropwise with stirring. Then stirring was continued for 3 hr, and the preparation was left standing overnight. Next water was added, and the solution was extracted with three 50-ml portions of ether. After evaporation of the ether, the residue was crystallized from alcohol, yielding 89 mg, mp 125–126°.

Anal. Calcd for $C_{16}H_{16}N_2O_8S$: C, 48.48; H, 4.07; N, 7.07. Found: C, 48.54; H, 4.26; N, 6.99.

3-O-Palmitoyl-α^4,α^5-isopropylidenepyridoxol.—α^4,α^5-Isopropylidenepyridoxol (1.0 g) was dissolved in 50 ml of dry pyridine, and palmitoyl chloride (3 ml) was added drop by drop with stirring and ice cooling over a period of 1 hr. The reaction mixture was stirred in the cold for 2 hr, and was next shaken in a "wrist action" shaker for 2 days. The pyridine solution was then evaporated to dryness under reduced pressure. The residue was crystallized from methyl alcohol, yielding 1.1 g (51.4%), mp 55–56°.

Anal. Calcd for $C_{27}H_{45}NO_4$: C, 72.48; H, 10.06; N, 3.01. Found: C, 72.51; H, 10.32; N, 3.10.

α^4-O-Palmitoylpyridoxol Hydrochloride.—3-O-Palmitoyl-α^4,α^5-isopropylidenepyridoxol (350 mg) was dissolved in a mixture of 0.01 *N* HCl (20 ml) and 95% ethyl alcohol (50 ml), and was then heated on a steam bath for 1.5 hr. The solution was next evaporated to dryness under reduced pressure, and the residue was crystallized from a methanol–ether mixture, yielding 0.31 g (89.3%), mp 149–150°.

Anal. Calcd for $C_{24}H_{42}NO_4Cl$: C, 64.93; H, 9.47; N, 3.15. Found: C, 64.95; H, 9.64; N, 3.49.

The free base was isolated by adding sodium bicarbonate until alkalinity was reached and was extracted with chloroform: mp 85–86°.

α^5-O-Palmitoyl-α^4,3-O-isopropylidenepyridoxol was prepared by the procedure described for 3-O-palmitoyl-α^4,α^5-O-isopropylidenepyridoxol, starting from α^4,α^5-O-isopropylidenepyridoxol (1.00 g); the yield was 1.3 g (61%), mp 43–44°. The compound was converted to its hydrochloride, which was then crystallized from a mixture of acetone and petroleum ether: mp 141–142° (lit.[8] mp 132.5–133.5°).

Anal. Calcd for $C_{27}H_{46}ClNO_4$: C, 67.01; H, 9.51; N, 2.89. Found: C, 66.82; H, 9.62; N, 2.83.

α^5-O-Palmitoylpyridoxol was prepared from α^5-O-palmitoyl-α^4,3-O-isopropylidenepyridoxol (0.30 g) by the same method as was used for the 4-O isomer, except that the compound was crystallized from methanol, yielding 0.25 g (91%), mp 99–100° (lit.[8] mp 72–76°).

Anal. Calcd for $C_{24}H_{41}NO_4$: C, 70.72; H, 10.14; N, 3.44. Found: C, 70.62; H, 10.14; N, 3.49.

3-O-Acetyl-α^4,α^5-isopropylidenepyridoxol.—α^4,α^5-Isopropylidenepyridoxol (3.0 g) was dissolved in a minimum quantity of dry pyridine, and 30 ml of acetic anhydride was added. After standing for 3 days, water was added, and the solution was extracted with three 50-ml portions of ether. The ether was evaporated, giving an oil. Tlc (10% MeOH–90% $CHCl_3$) indicated the presence of a substantial amount of the starting material (R_f 0.45), as well as the acetate (R_f 0.89). The starting material was removed by redissolving the oil in ether and extracting the solution with five 25-ml portions of 2% aqueous sodium hydroxide solution and then with three 25-ml portions of water. Evaporation of the ether gave an oil (3.47 g), which was now free of starting material.

The picrate was obtained in ethanol, and was recrystallized from ethanol: yellow needles, mp 163°.

Anal. Calcd for $C_{19}H_{20}N_4O_{11}$: C, 47.50; H, 4.20; N, 11.66. Found: C, 47.49; H, 4.20; N, 11.71.

α^4-O-Acetylpyridoxol.—The oily 3-O-acetyl-α^4,α^5-isopropylidenepyridoxol (3.47 g) was heated in 10% aqueous acetic acid (150 ml) on a steam bath for 1 hr. Evaporation *in vacuo* gave an oil, which was treated several times with absolute alcohol to remove acid and water. Tlc (10% MeOH–90% $CHCl_3$) of this oil produced four spots. The oil was dissolved in a small amount of ethanol and was cooled with dry ice; 241 mg of crystalline material was obtained, mp 139–146°. Recrystallization of the material from ethanol yield 203 mg of crystals, mp 149–150°; after evaporation of the mother liquor, a crop of different crystals was obtained, mp 152–154°; tlc with 1:9 methanol–chloroform gave R_f 0.68 and 0.34, respectively. The lower R_f material was shown to be identical to pyridoxol by tlc, infrared, and nmr. The higher R_f material was recrystallized from ethanol: mp 162–165°; after drying at 60° *in vacuo*, mp 155–157°; nmr, (DMSO-d_6) CH$_3$ (acetyl) −125, 2-CH$_3$ −143, 4-CH$_2$ −309, 5-CH$_2$ −287, C$_6$-H −478.

Anal. Calcd for $C_{10}H_{13}NO_4$: C, 56.86; H, 6.20; N, 6.63. Found: C, 56.62; H, 6.26; N, 6.54.

Registry No.—I (R_3 = R_4 = R_5 = H), 65-23-6; I (R_3 = Me; R_4 = COPh; R_5 = H), 14210-65-2; I (R_3 = Me; R_4 = H; R_5 = COPh), 14210-66-3; I (R_3 = CH_3SO_2; R_4 = H; R_5 = COPh), 14210-67-4; I (R_3 = H; R_4 = R_5 = COPh), 14210-68-5; I (R_3 = CH_3SO_2; R_4 = R_5 = COPh), 14210-69-6; I (R_3 = R_4 = R_5 = COPh), 14210-70-7; I (R_3 = R_4 = COPh; R_5 = H), 14210-71-0; I (R_3 = R_4 = COPh; R_5 = H) hydrochloride, 14210-72-1; I (R_3 = CH_3SO_2; R_4 = *p*-$O_2NC_6H_4CO$; R_5 = H),

14210-73-2; III (R = $C_{15}H_{31}$), 14320-27-5; III (R = $C_{15}H_{31}$) hydrochloride, 14210-74-3; IV (R = $C_{15}H_{31}$), 14210-75-4; VI (R = Ph), 14210-76-5; VI (R = Ph) hydrochloride, 14210-77-6; VI (R = p-$O_2NC_6H_4$), 14210-78-7; VI (R = $C_{15}H_{31}$), 14320-31-1; VI (R = CH_3), 14213-49-1; VI (R = CH_3) picrate, 14213-50-4; VIII (R = Ph), 5223-10-9; VIII (R = Ph) hydrochloride, 14210-83-4; VIII (R = p-$O_2NC_6H_4$) hydrochloride, 14320-28-6; VIII (R = $C_{15}H_{31}$), 14210-84-5; VIII (R = $C_{15}H_{31}$) hydrochloride, 14210-85-6; VIII (R = CH_3), 14210-86-7.

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Bromination of the 3,6-Endoxo-Δ^4-Tetrahydrophthalic Anhydride System. Stereochemistry and Mechanism of the Reaction

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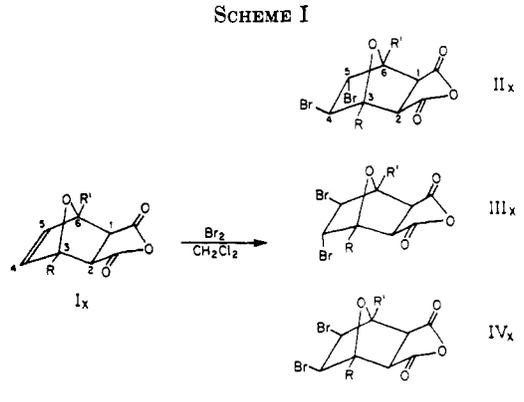
The bromination reactions of two different methyl derivatives of the 3,6-endoxo-Δ^4-tetrahydrophthalic anhydride have been studied. On the basis of the stereochemical course of the reaction, the effects of methyl substitution upon it are discussed. The validity of a mechanism proposed for this reaction in an earlier work is questioned.

Some years ago, Berson and Swidler studied the bromination reaction of *exo-cis*-3,6-endoxo-Δ^4-tetrahydrophthalic anhydride (Ia)[2] in various solvents. In that elegant work, the stereochemistry of bromine addition to the bicyclic olefin Ia was clearly established and the authors put forward the hypothesis of the existence of a special free-radical mechanism to rationalize the unusually high proportion of *cis*-bromination product IVa formed in the reaction. In their scheme, a concurrent ionic mechanism was responsible for the formation of at least part of the *trans*-bromination products IIa–IIIa, which constitute a racemate.

We decided to study further this reaction with a dual purpose—first, to determine the influence that methyl substitution might have on the stereochemical course of this bromination reaction and, second, to continue the study of long-range couplings in the nmr spectra of bicyclic molecules which are of general interest to us.

The bromination of 3,6-endoxo-Δ^4-tetrahydrophthalic anhydride (Ia) and of the 3-methyl (Ib) and 3,6-dimethyl (Ic) derivatives was carried out in methylene chloride (see Scheme I). All products obtained were analyzed by nmr and elemental analysis and the results are summarized in Table I. The reported values for the bromination of Ia are the ones obtained in this work and they are substantially the same as the ones reported by Berson.[1] Table II contains all the information relative to the nmr spectra for every one of the dibromo compounds obtained.

SCHEME I



x : a (R = R' = H)
x : b (R = CH_3, R' = H)
x : c (R = R' = CH_3)

TABLE I

BROMINATION OF OXOBICYCLIC OLEFINS

Olefin	Total yield, %	Product distribion, %		Stereochemical course
Ia	86	IIa ≡ IIIa	65.1	*trans*
		IVa	34.9	*cis*
Ib	83	IIb	22.4	*trans*
		IIIb	60.3	
		IVb	17.3	*cis*
Ic	80	IIc ≡ IIIc	100	*trans*
		IVc	0	*cis*

Discussion of the Results

The values for product distribution collected in Table I clearly indicate that methyl substitution at the bridgehead of the oxobicyclic system Ia has a marked effect on the stereochemistry of bromine addition. When a methyl group is located on the bridgehead carbon atom (Ib), the proportion of *cis*-bromination product is lowered by a factor of 0.5 with respect to the unmethylated substrate Ia and no *cis* bromination occurs when the two bridgehead carbon atoms are methyl substituted (Ic). Also, from the two *trans*-bromination products of possible formation in the case of Ib, the one with the bromine at the *endo* position on the carbon atom α to the bridgehead carrying the methyl substituent (IIIb) predominates by a factor of 2.4 over the other isomer (IIb). These facts seem to indicate that regardless of the mechansim, some important steric

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(2) J. A. Berson and R. Swidler, *J. Am. Chem. Soc.*, **76**, 4060 (1953).