the resulting solution was strongly alkaline. This solution was then exhaustively extracted with ether, using a continuous liquid-liquid extraction apparatus.

The ether extract was dried over magnesium sulfate, treated with decolorizing carbon, and then filtered. The filtrate was carefully concentrated and the residual oil fractionated. Compound XXIII was isolated directly as a solid residue and recrystallized from methanol.

*Acknowledgment.* The authors wish to express sincere thanks to Prof. F. M. Beringer and Dr. J. G. Lombardino for helpful discussions. The technical assistance of Mr. H. Talts and Mr. R. Parla is also gratefully acknowledged.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF FLORIDA]

# Pyrazolines. V. Application of Nuclear Magnetic Resonance Spectrometry to Structure Determinations[1]

WALLACE S. BREY, JR., AND W. M. JONES

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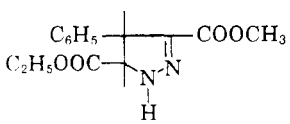
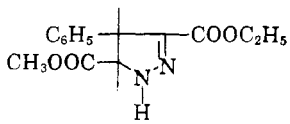
The structures of the two isomeric mixed methyl, ethyl esters of 4-phenyl-2-pyrazoline-3,5-dicarboxylic acid have been elucidated by comparison of their NMR spectra with appropriate model compounds. Interpretation of various features of the NMR spectra is discussed.

In the latter part of the nineteenth century, Buchner and his co-workers[2] made the interesting observation that the reactions of methyl diazoacetate with ethyl cinnamate and ethyl diazoacetate with methyl cinnamate led to two different isomeric 2-pyrazolines to which they assigned structures I and II, respectively. Although they did

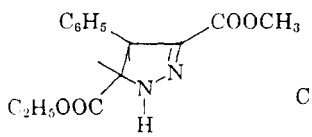


I

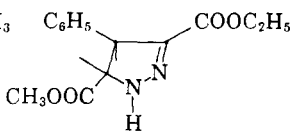


II

present compelling evidence for gross structures for these two isomers, they were not able to demonstrate conclusively the positions of the ester methyl and ethyl groups. In addition, no attempt was made to assign geometrical configurations to these two products.



I a



II-a

Geometrical configurations Ia and IIa have recently been assigned[1] as a result of an examination of the decomposition products of these two materials and the application of the rule[3] that "the geometrical configuration of the primary cyclopropane resulting from the decomposition of a 2-pyrazoline is determined by the relative thermodynamic stabilities of the intermediate 1-pyrazolines."

There still remained, however, the problem of distinguishing between the two isomers Ia and IIa. This distinction has been effected by comparing the proton magnetic resonance spectra of these two compounds with four model compounds.

## EXPERIMENTAL

The mixed methyl, ethyl esters were synthesized by the method of Buchner and co-workers[2]; I, m.p. 106–106.5°, reported m.p. 107°; II, m.p. 75–76°, reported m.p. 76°. The methyl ester of 4-phenyl-2-pyrazoline-3-carboxylic acid (III) was prepared by the method of von Pechman and Burkhard[4]; m.p. 127–128°, reported m.p. 128°. The ethyl ester of 4-phenyl-2-pyrazoline-3-carboxylic acid (IV) was synthesized according to von Auwers and Cauer[5]; m.p. 99.5–100.5°, reported m.p. 100–100.5°. The dimethyl ester (V) of 4-phenyl-2-pyrazoline-3,5-dicarboxylic acid and the diethyl ester (VI) of this acid were prepared by the method of Buchner and co-workers;[2a,6] dimethyl ester, m.p. 103–104°, reported m.p. 105°; diethyl ester, m.p. 78–78.5°, reported m.p. 79°. Assignment of the *trans* configuration to both the dimethyl and the diethyl esters is based on the decomposition products of these two materials[3, 6, 7] and the recent demonstration that tautomeric equilibrium is not attained prior to nitrogen loss in the thermal decomposition of 3,5-dicarboalkoxy-2-pyrazolines.

NMR spectra were determined for the mixed esters and the model compounds in saturated solutions in carbon tetrachloride. A Varian 4300-2 high-resolution spectrometer, operating at 56.4 megacycles, was employed. Shifts were measured with respect to benzene as an internal reference, using sidebands applied by a calibrated audio oscillator. The amount of benzene added to the solutions was adjusted to give a peak of the same size as that of the phenyl group of the sample.

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(1) For the previous paper, see W. M. Jones, *J. Am. Chem. Soc.*, **82**, 3136 (1960).

(2)(a) E. Buchner and H. Dessauer, *Ber.*, **26**, 258 (1893); (b) E. Buchner and C. von der Heide, *Ber.*, **35**, 31 (1902).

(3) W. M. Jones, *J. Am. Chem. Soc.*, **80**, 6687 (1958); **81**, 5153 (1959).

(4) H. von Pechman and E. Burkhard, *Ber.*, **33**, 3595 (1900).

(5) K. von Auwers and E. Cauer, *Ann.*, **470**, 284 (1929).

(6) E. Buchner, *Ber.*, **21**, 2637 (1888).

(7) E. Buchner and H. Dessauer, *Ber.*, **25**, 1147 (1892).

TABLE I

NMR CHEMICAL SHIFTS OF SUBSTITUTED PYRAZOLINES

Values are in parts per million; positive shifts are to higher magnetic field

| Compound | Substituent Group 3 | Substituent Group 5 | H in $OCH_3$ | H in $CH_2$ of $OC_2H_5$ | H in $CH_3$ of $OC_2H_5$ | H in $C_6H_5$ | H on Ring Carbons |
|---|---|---|---|---|---|---|---|
| I | $COOCH_3$ | $COOC_2H_5$ | 3.60 | 3.03 | 5.96 | 0.043 | 2.75<br>3.03 |
| II | $COOC_2H_5$ | $COOCH_3$ | 3.56 | 3.19 | 6.08 | 0.055 | 2.75<br>2.93 |
| III | $COOCH_3$ | H | 3.63 | — | — | 0.108 | [a] |
| IV | $COOC_2H_5$ | H | — | 3.14 | 6.06 | 0.078 | [a] |
| V | $COOCH_3$ | $COOCH_3$ | 3.49<br>3.60 | — | — | 0.041 | 2.71<br>3.01 |
| VI | $COOC_2H_5$ | $COOC_2H_5$ | — | 3.01<br>3.14 | 5.98<br>6.08 | 0.046 | 2.71<br>2.94 |

[a] Splitting by H in position 5 prevented analysis.

## RESULTS AND DISCUSSION

In Table I are presented the chemical shifts of the hydrogen atoms in the six compounds, expressed in parts per million displacement of the resonance upfield from the reference (benzene).

The resonance of the hydrogen atoms in an $OCH_3$ group is a single sharp peak, while each ethyl group shows the usual 4 and 3 multiplets with coupling constants of 7.1–7.2 cycles. The two hydrogen atoms attached to the ring carbons show a characteristic nonequivalence quartet, which was analyzed in the usual manner to give the chemical shifts shown. In many of the spectra, this quartet overlapped that of the methylene hydrogens of the ethyl group, but the differences in relative spacings within the two types of quartet permitted unambiguous assignments to be made.

The assignments of structures I and II, as indicated in the table, to the high and low melting isomers, respectively, may be confirmed by examination of the chemical shifts of the H atoms in alkoxy groups. The carbomethoxy group in the 3 position of III, a known compound, has a value of 3.63. In compound V, there are carbomethoxy groups in both 3 and 5 positions; the respective chemical shifts may be assigned as 3.60 and 3.49. The value of 3.60 appears also in I, corresponding to a carbomethoxy group in the 3 position; in II, the lower value of 3.56 for the same substituent group is consistent with the location of this group in the 5 position. Even more striking is the distinction between the chemical shifts of the carboethoxy hydrogens in the two positions. In IV, the methylene protons appear at 3.14, a value also found for one of the two groups in compound VI, and comparable to the value 3.19 for compound II. In contrast, the other methylene hydrogens in the diethyl ester, VI, have a chemical shift at 3.01, which corresponds to the value of 3.03 in compound I, indicating that the ethyl group in the latter is in the 5 position. In parallel fashion, the resonances for the methyl hydrogens in the ethyl esters appear at 6.06–6.08 for the 3 position and 5.96–5.98 for the 5 position.

There are two possible factors that can explain the result that the resonances for a carboalkoxy group *always appear at higher field when the group is in the 3* position than when it is in the 5 position. The first of these is based upon the conjugation of the carbonyl group of the position 3 substituent with the ring double bond, which would be expected to reduce the conjugation of the unshared electrons on the ether oxygen of the ester unit with the carbonyl group and therefore increase the electron density on the ether oxygen and in turn on the alkyl group. It is not expected, however, that this effect would be transmitted to the extent it is to the methyl group in the ethyl radical. The second factor, which would apply particularly to this group, is related to the fact that in the *trans* configuration of these compounds, the alkyl group of the ester in position 3 has a higher probability than does the group in position 5 of being located in front of the face of the phenyl ring. It is known that the ring current effect in the aromatic ring shifts resonances of groups to higher field when the groups are in this sort of location with respect to the ring.[8]

Chemical shifts of the hydrogens attached to carbons in the pyrazoline ring display an interesting pattern. The resonance at higher field appears at 3.01–3.03 when there is a carbomethoxy group in position 3 but at 2.93–2.94 when there is a carboethoxy group in this position. The resonance at lower field appears at 2.71 when positions 3 and 5 have the same substituent group but at 2.75 for the mixed esters. This indicates that there is some change in the precise ring geometry related to the presence of the alkyl groups in the esters.

Finally, the position of the phenyl resonance is of interest. In compounds III and IV, the phenyl group has the greatest freedom of rotation about its bond to the ring, and the chemical shift has the highest positive value. The other four compounds

(8) J. B. Hyne, *J. Am. Chem. Soc.*, 81, 6058 (1959).

listed in Table I have about the same rotational freedom for the phenyl, slightly less than that in III and IV, and the chemical shifts of the phenyl protons fall in a relatively narrow range, somewhat below III and IV. In addition to the results reported in Table I, we have obtained spectra of solutions of oils isolated from the mother liquors resulting from filtration of I and II from their crude reaction mixtures. Evidence has been presented[1] that these mother liquors are rich in the *cis*-4-phenyl-3,5-dicarboalkoxy-2-pyrazolines. In these spectra there are resonances reported for either compound I or II as well as additional bands. The latter are believed to be characteristic of the *cis* isomers, since the phenyl peaks fall at lower fields, one chemical shift being negative. In the *cis* isomer, the phenyl group is wedged between the two substituents. The shift of the resonance of the phenyl group to lower magnetic field is therefore attributed to steric effects, very probably a direct "repulsive unshielding" of the phenyl protons.[9]

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(9) G. V. D. Tiers, *J. Am. Chem. Soc.*, **78**, 2914 (1956).

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[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, YALE UNIVERSITY SCHOOL OF MEDICINE]

# Potential Deoxyribonucleic Acid Cross-linking Agents. 8,8′-Bispurines[1,2]

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*Received September 28, 1960*

As part of a program involving the preparation of compounds, incorporation of which could result in interhelical cross-linking of deoxyribonucleic acid, a group of 8,8′-bispurines connected by a four-carbon chain has been synthesized. 8,8′-Bisdihydropurinyl was obtained by the reaction of 5,6-diaminopyrimidine with glyoxal.

The synthesis of 8,8′-bispurines was undertaken as part of a program aimed at preparing compounds specifically capable of cross-linking deoxyribonucleic acid. Deoxyribonucleic acid is now generally accepted as having the structure of a double helix,[3] the twin strands of which are held together by hydrogen-bonding between adenine and thymine or between guanine and cytosine. This interaction involves groups attached to the 2- and 4-positions of the pyrimidines and the 2- and 6-positions of the purines.

It is possible to consider either intra-helical or extra-helical cross-linking of deoxyribonucleic acid. In the former case, the groups responsible for the hydrogen-bonding between purine and pyrimidine rings could be so modified as to lead to unusually strong interaction within the double structure inhibiting its replication. It seems possible that 6-thioguanine, which is incorporated into deoxyribonucleic acid,[4] exerts its carcinostatic effects by forming stronger hydrogen bonds through its highly polarized C–S group with cytosine than does guanine. Inter-helical cross-linking would imply interaction between adjacent double helices. The antitumor activity of alkylating agents, which has been postulated[5] as being due to cross-linking between the phosphate groups located on the outer shell of deoxyribonucleic acid, might provide an example of possible inter-chain interaction.

Attempts have been made to synthesize compounds the incorporation of which could result in specific cross-linking between adjoining molecules of deoxyribonucleic acid.[6]

In one approach to this problem a group of 8-purinyl nitrogen mustards has been synthesized,[7] in another approach it was decided to prepare bispurines. Double incorporation of these—or of their ribonucleosides or deoxyribonucleosides—into deoxyribonucleic acid would result in cross-linking of the inter-helical type.

The 8-position was chosen for the attachment of the rather bulky groups being introduced here, since there is good evidence,[8] that hydrogen-bonding between purines and pyrimidines is necessary for deoxyribonucleic acid synthesis. Accordingly, for incorporation of the bis-compounds to take place it would seem desirable for the cross-linking groups to extend radially toward the periphery of the double helix without interfering with intra-helical hydrogen-bonding. For this purpose the 8-position of purines and the 5-position of cytosine or the methyl group of thymine would seem to be the most suitable points of attachment.

As a first approach to the synthesis of 8,8′-bispurines, the condensation of 5,6-diaminopyrimi-

(1) Part of this material was presented at the meeting of the Medicinal Chemistry Section of the American Chemical Society, Cleveland, Ohio, April, 1960, 9-N.

(2) This work was supported, in part, by a grant (CY-3937) from the National Institutes of Health, Public Health Service.

(3) J. D. Watson and F. H. C. Crick, *Nature*, **171**, 737 (1953).

(4) G. A. LePage, *Cancer Res.*, 20, 403 (1960).

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(6) H. G. Mautner, *Yale J. Biol. Med.*, **32**, 323 (1960).

(7) S. H. Chu, J. E. Harris, and H. G. Mautner, *J. Org. Chem.*, **25**, 1759 (1960).

(8) A. Kornberg, *Science*, **131**, 1503 (1960).