TABLE I

$[NiX_2(PEtPh_2)_2]$	Dec. temp., °C.	Color	μ_{eff}(B.M.)	Dipole moment (Debye units)	Molecular weight[a] Calcd.	Found
(I) X = Cl	146–151	Dark red	Diamag.	3.2	558	550[b], 535[c]
(II) X = Br[d]	165–175	Dark green	3.20	5.9	647	558[b], 625[c]
(III) X = I	127–138	Brown-red	3.10	7.5	741	745[b], 730[c]
(IV) X = Br	160–175	Dark brown	Diamag.	...	647	575[b]

[a] The molecular weight values (except for (II) and (IV) in benzene) were obtained by boiling-point elevation and were extrapolated to infinite dilution; there was usually a small *decrease* (< 10%) in the observed molecular weight with increasing concentration. (II) and (IV) were measured in 1.3% benzene solution at 37° using a vapor pressure osmometer. [b] Benzene solution. [c] Chloroform solution. [d] First reported in ref. 4.

by the isomorphism of (II) (X-ray powder pattern) with the tetrahedral complexes $[MBr_2(PEtPh_2)_2]$ (M = Co, Zn). In solution, the properties of (I), (II) and (III) are similar to those of the corresponding complexes of Ph_3P and Ph_2-*n*-Bu-P in that they are monomeric, and have high dipole moments and similar spectra in the range 400–1000 mμ. Thus, even $[NiCl_2(PEtPh_2)_2]$ apparently forms a certain amount of polar, paramagnetic species in solution.

These results suggested that the solutions may indeed contain a mixture of geometric isomers and that any equilibrium between them may be greatly affected by the experimental conditions. Qualitative experiments with (II) show that the diamagnetic (red) form is favored by nonpolar solvents and low temperatures and that the paramagnetic (green) form is favored by polar solvents. Thus, (II) gives a dark red solution in carbon disulfide, in which, on cooling to −78°, slowly deposits red crystals (IV), which are dark brown after isolation and are isomeric with (II).

Anal. Calcd. for $C_{28}H_{30}Br_2NiP_2$: C, 52.0; H, 4.7; Br, 24.7; Ni, 9.1; P, 9.6. Found; C, 51.7; H, 4.7; Br, 24.85; Ni, 9.9; P, 9.4. (IV) has an X-ray powder pattern distinct from that of (II), but very similar to that of $[PbBr_2(PEtPh_2)_2]$; this observation and the diamagnetism show that (IV) is square planar in the crystal. The spectrum of (IV) in the solid state is consistent with this and shows a general resemblance to the spectrum of *trans*-$[NiBr_2(P$-*n*-$Bu_2Ph)_2]$[2]; in particular, (IV) does not absorb in the region 800–1000 mμ, in contrast to (II), which shows a band at 862 mμ, and to other tetrahedral compounds of this type.[2] In benzene solution, the spectra of (II) and (IV) are indistinguishable and both show absorption at 880 mμ (ε = 130).

On standing at room temperature, or more rapidly on heating, (IV) gradually turns green, this change being accompanied by an increase in magnetic moment, a constant value of 3.20 B.M. being reached at about 90°. The X-ray powder pattern of the resulting green solid is identical with that of (II). Some salicylaldimine[5] and 1,3-diketone[6] complexes of bivalent nickel show apparently similar temperature-dependent behavior and that usually has been interpreted satisfactorily in terms of square planar–octahedral isomerism, the octahedral structure being achieved by polymerization.[5–7] The evidence presented above, however, shows that green $[NiBr_2(PEtPh_2)_2]$ is tetrahedral (or approximately so) in the solid state and is not polymerized in solution.

(II) and (IV) thus represent the first example of square planar–tetrahedral isomerism among complexes of nickel(II), or indeed of any metal. Further examples have been found among compounds of the type $[NiX_2(PRPh_2)_2]$ and both forms have been isolated in the solid state when X = Br(R = *n*-Pr, *i*-Pr, *n*-Bu) and X = Cl(R = *n*-Bu). We consider that these results afford strong, albeit circumstantial, evidence that, in solution, $[NiX_2(PRPh_2)_2]$ (R = alkyl, Ph) exist as equilibrium mixtures of the square planar and tetrahedral somers.

The authors are indebted to Dr. L. Vaska and Mr. R. E. Rhodes for the measurement of the magnetic moments, and to Dr. R. D. Feltham for discussions and suggestions.

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RECEIVED MARCH 22, 1962

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(5) C. M. Harris, S. L. Lenzer and R. L. Martin, *Aust. J. Chem.*, **14**, 420 (1961).

(6) J. F. Fackler, Jr., and F. A. Cotton, *J. Am. Chem. Soc.*, **83**, 3775 (1961).

(7) R. H. Holm, *ibid.*, **83**, 4683 (1961), and references therein.

APPLICATION OF MASS SPECTROMETRY TO STRUCTURE PROBLEMS.[1] VI. NUCLEOSIDES[2]

Sir:

The identification, characterization, and structure determination of nucleosides and related compounds is to a certain extent hampered by their high melting points, their polarity, and insolubility in most organic solvents. Migration in various chromatographic or electrophoretic systems, combined with ultraviolet spectroscopy, is most often used to characterize such molecules. We have, therefore, investigated the applicability of mass spectrometry[3] in this field, although the very low volatility of these compounds would at first seem to preclude this approach. Instead of converting these substances into more volatile derivatives, as in the case of amino acids[4] and peptides,[5] we have utilized free nucleosides, subliming them directly into the ionizing electron beam of the mass spectro-

(1) Part V: K. Biemann and M. Friedmann-Spiteller, *J. Am. Chem. Soc.*, **83**, 4805 (1961).

(2) This investigation was supported by grants from the National Institutes of Health (RG-5472) and the National Aeronautics and Space Administration (NsG 211-62). We wish to thank Dr. Jack J. Fox for samples of synthetic nucleosides and Mr. M. Munroe for invaluable help with the instrumentation.

(3) For a discussion of organic mass spectrometry and the interpretation of spectra see K. Biemann, *Angew. Chemie*, **74**, 102 (1962); *Intern. Ed.*, **1**, 98 (1962); K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962.

(4) K. Biemann, J. Seibl and F. Gapp, *J. Am. Chem. Soc.*, **83**, 3795 (1961).

(5) K. Biemann, F. Gapp and J. Seibl, *ibid.*, **81**, 2274 (1959); K. Biemann, *Chimia* (Switz.), **14**, 393 (1960).

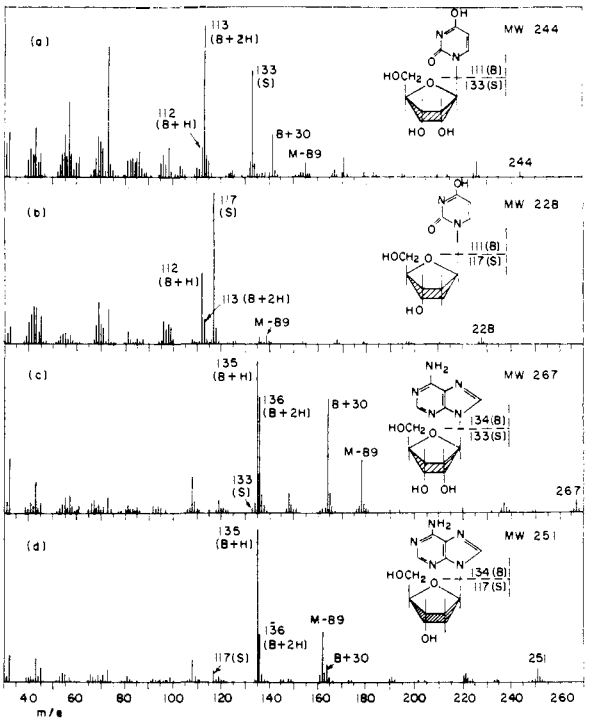


Fig. 1.—Mass spectra of uridine (a), deoxyuridine (b) adenosine (c) and deoxyadenosine (d).

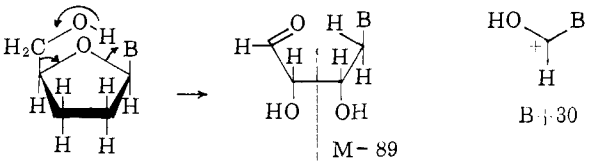
meter,[6] mainly for two reasons: first, only extremely small amounts[7] are required using this technique; second, the time- and material-consuming chemical pretreatment is avoided.

Mass spectra of a number of nucleosides (those shown in Fig. 1 and thymidine, guanosine, deoxyguanosine, isopropylideneadenosine, isopropylidineguanosine; the 1-β-D-arabinofuranoside, 1-β-D-lyxofuranoside and 1-(5′-deoxy)-β-D-lyxofuranoside of uracil,[8] and the O,N-perdeuterio analogs[9] of some of them) have been obtained and were found to permit the identification of the base and to yield considerable information about the sugar moiety. These conclusions are based on these characteristics of the spectra:

The pyrimidine or purine residue (B, its mass equals the mol. wt. of the free base minus 1) gives rise to a prominent peak, sometimes the most intense one, at masses B + 1 and B + 2 in a rearrangement process involving one and two hydrogen atoms, respectively. These are removed preferentially from hydroxyl groups as judged from the spectra of the O,N-perdeuterio derivatives. The double rearrangement is more facilitated in ribosides than in 2′-deoxyribosides [10] and is more pronounced in pyrimidine derivatives (see Fig. 1-a and 1-d). Thus the relative intensities of B + 1 *vs.* B + 2 permit differentiation between ribosides and 2′-deoxyribosides, and probably between pentosides and 2′-deoxypentosides in general, while their mass indicates the identity of the base.

In addition, the mass of the "sugar fragment" (S) is found at *m/e* 117 in deoxypentosides and at *m/e* 133 in pentosides. Its intensity is much lower if the base is a purine rather than a pyrimidine since a purine, having a higher electron density, competes with the sugar moiety more efficiently for the positive charge than can a pyrimidine. The ribose fragment generally is less abundant than the 2′-deoxy fragment. Combination of these effects leads to the extreme intensity differences of the sugar peaks in Figs. 1b and c.

Peaks at lower mass (< *m/e* 100) are due mainly to fragments of the sugar moiety; more interesting are those of higher mass: at M (mol. wt.), M-18 (loss of H_2O), M-89 and B + 30. The loss of 89 m.u. is interpreted as shown:



Thus, the 5′-deoxynucleoside fails to exhibit a peak at M-89. The nature of the fragment B + 30 is as shown above. The rearranged hydrogen seems to be abstracted preferably from 2′-OH in view of the low intensity of the peak in the 2′-deoxy derivatives (Fig. 1b and d). The spectra of the O,N-perdeuterio nucleosides and isopropylidene derivatives (which are also of higher volatility) are in agreement with these interpretations.

Differences in the steric arrangement of the hydroxyl groups give rise to marked variations in the intensity of some peaks. Epimers can thus be distinguished from one another, but the examples thus far available have not permitted a detailed interpretation. The spectra obtained after conversion of the sample into an isopropylidene derivative may be helpful in such cases.

It would seem that mass spectrometry has considerable potentialities for the characterization and determination of the structure of nucleosides and related compounds, especially in the synthesis of analogs, a field which has become of great interest in recent years.

Beyond this, the results presented indicate that detailed structural information can be obtained about organic molecules of remarkably low vola-

(6) Spectra were determined with a Bendix Time-of-Flight mass spectrometer (Model 12-107). Samples were introduced using the No. 843 hot filament system, considerably modified to permit vaporizing the sample about 1 cm. from the ionizing electron beam. Spectra were recorded on a Honeywell 1508 Visicorder in 30 to 60 sec. Reliable mass identification was accomplished either by adding a calibration mixture (bromoform, *p*-dibromobenzene, and hexachlorobutadiene) or using the proportionality of $\sqrt{m}$ and flight time, or both.

(7) About 20 to 50 μg. of material was used for the spectra discussed here. Slight modifications of the sample handling and recording techniques will permit the use of much smaller amounts because of the inherent sensitivity of the mass spectrometer.

(8) R. Fecher, J. F. Codington, and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 1889 (1961).

(9) These were prepared simply by dissolving the sample, after placing it into a small capillary which fits into the introduction system (ref. 6), in a few μl. of D_2O and evaporation of the solvent in a desiccator. Since the sample is vaporized into the electron beam without a chance of colliding with ordinary water adsorbed on surfaces, deuterium bound to N and O is retained, in contrast to its partial loss if a conventional inlet system were employed. Aside from information regarding the fragmentation process, such spectra may yield valuable information about the number of active hydrogens present in the entire molecule, or fragments thereof, if its structure is to be deduced.

(10) This effect seems to be related only to the absence of a 2′-hydroxy group since the 5′-deoxylyxoside also exhibits a prominent B + 2 peak.

tility, an area previously thought to be beyond the realm of mass spectrometry.

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THE RESPONSE OF THE ANEMIC AND DYSTROPHIC MONKEY TO TREATMENT WITH COENZYME Q

Sir:

The anemia and muscular dystrophy that develop in rhesus monkeys (*Macacus mulatta*) supplied with a purified diet low in vitamin E is rapidly progressive, and has been found to lead to death invariably unless α-tocopherol is given.[1] The specific metabolic defect(s) responsible for these abnormalities remains unknown, although various alterations in metabolism in the deficient monkey have been observed.[1-3] These metabolic deficiencies resulted after maintenance of the monkey for many months, and the deficiencies and the response to α-tocopherol naturally were interpreted solely on the basis of vitamin E. Most of these studies were carried out before the discovery and elucidation of coenzyme Q (ubiquinone).

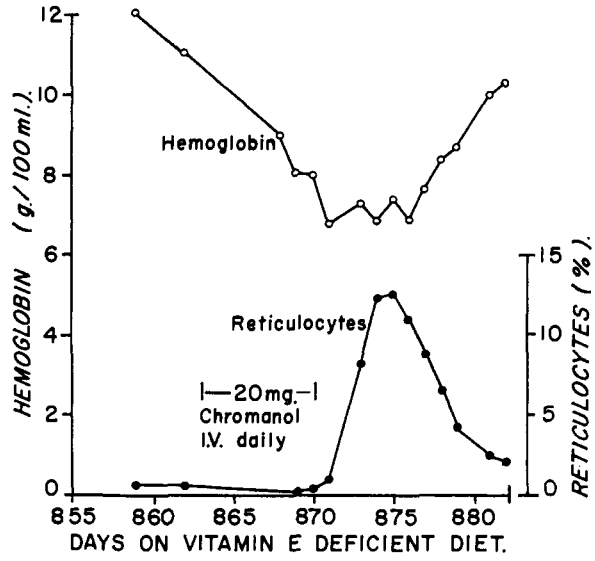


Fig. 1.—The response of a deficient, anemic monkey (no. 223) to the 6-chromanol of hexahydrocoenzyme Q_4.

Today, new knowledge is available on the isolation of coenzyme Q from natural sources, and on the role of coenzyme Q with succinoxidase and cellular respiration. When one compares the organic structures of certain members of the vitamin E and coenzyme Q groups, such as α-tocopherol (I) and the 6-chromanol[4] of hexahydrocoenzyme Q_4 (II), it is seen that they are identical, except for the interchange of two methyl and two methoxy groups in the 7 and 8 positions. Although only quinones of the coenzyme Q group (coenzyme Q_{10} has been isolated from tissue of the rhesus monkey)[5]

(1) J. S. Dinning and P. L. Day, *J. Exp. Med.*, **105**, 395 (1957).
(2) J. S. Dinning and P. L. Day, *J. Nutr.*, **63**, 393 (1957).
(3) J. S. Dinning and P. L. Day, *J. Biol. Chem.*, **233**, 240 (1958).
(4) C. H. Shunk, N. R. Trenner, C. H. Hoffman, D. E. Wolf, and K. Folkers, *Biochem. and Biophys. Res. Comm.*, **2**, 427 (1960).
(5) Unpublished data of Gale, Page and Folkers.

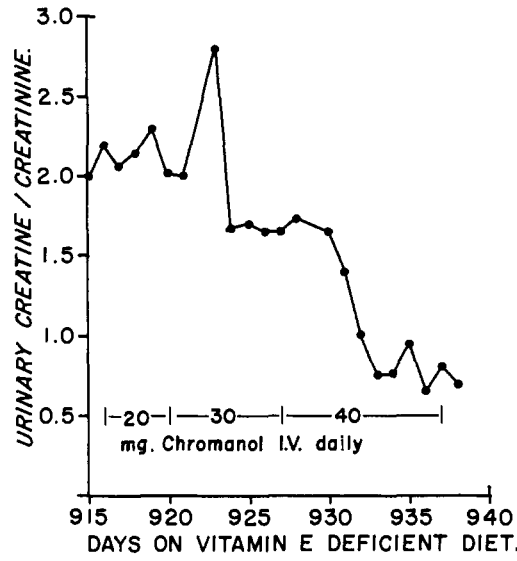
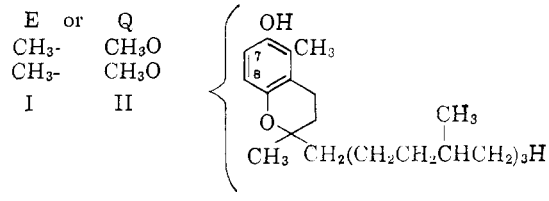


Fig. 2.—The response of a dystrophic deficient monkey (no. 218) to the 6-chromanol of hexahydrocoenzyme Q_4.

have been characterized from tissue extracts to date, their reductively cyclized chromanols have already been shown to have biological activity, and are being studied as possible enzymic forms of coenzyme Q. Two examples of comparable biological



activity of α-tocopherol and members of the coenzyme Q group are fetal resorption in rats[6] and the maintenance of chick sperm motility.[7] On the basis of such activities in both the E and Q groups, the known biochemistry of coenzyme Q as well as related organic structures, we have studied the responses of the anemic and dystrophic monkey to the treatment with the 6-chromanol of hexahydrocoenzyme Q_4 (II), as the first Q-form compound to test.

The handling of the animals and the purified diets, which are deficient of vitamin E, have been described.[1,8] Two animals were used. One (No. 223) received a low-fat diet and the other (No. 218) received a diet that contained 8% of lard, and was supplemented with 3 g. of cod liver oil daily. Both diets were supplied *ad libitum*. The first monkey had one prior test with α-tocopherol, and a good response was obtained. In the monkey that received fat in the diet, only a partial remission (as usual) had been obtained with N,N'-diphenyl-*p*-phenylenediamine. Each monkey was in a definite relapse and appeared near death before treatment with II. They were injected intravenously with a

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(7) A. C. Page, Jr., M. C. Smith, P. H. Gale, D. Polin, and K. Folkers, *Biochem. and Biophys. Res. Comm.*, **6**, 141 (1961).
(8) C. D. Fitch, J. S. Dinning, L. A. Witting and M. K. Horwitt, *J. Nutr.*, **75**, 409 (1961).