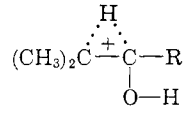
In the original work[1] the values of the $k_3/(k_{-2} + k_3)$ terms were assigned on the basis of reasonable assumptions coupled with data available in the literature. The conclusion was then reached that the isotope effect occurred solely in the ion-forming step (k_2) and was consistent with hydrogen participation in this step.

The data from the hydrolysis and rearrangement of the methoxyalcohols now makes these terms directly accessible. Consideration of the reaction scheme for the methoxyalcohols reveals that the ratio of k_{-2}/k_3 can be determined from the initial rates of formation of glycol and carbonyl product. It can also be seen that these values are simply the ratios of the appropriate values of a from the equations relating the percentage of each compound formed as a function of time. The values of k_{-2}/k_3 and $k_3/(k_{-2} + k_3)$ for the two methoxyalcohol systems are given in Table I.

The postulation of an open carbonium ion as the reaction intermediate allows one to make a reasonable calculation for the experimental isotope effect in the pinacol rearrangement. One may assign the following values to each discrete step in the above scheme: $k_2^H/k_2^D = 1.2$, a normal value for hyperconjugative stabilization of a carbonium ion[9]; $k_{-2}^H/k_{-2}^D = 1.2$, as demanded by the principle of microscopic reversibility; $k_3^H/k_3^D = 3$, a reasonable value for the hydride shift[10]; and $k_{-2}/^Hk_3^H = 2.6$, from our experimental determination. Using the equation for the experimental isotope effect and these values, an over-all isotope effect of *ca.* 2.5 may be calculated for the rearrangement of 2-methyl-1,2-propanediol and its deuterated analog. This value is not far from the observed value of 2.1. The calculation is instructive as it shows that the experimental isotope effect would arise mainly due to differences in the k_{-2}/k_3 terms. Based on the open ion postulate a value of k_{-2}^D/k_3^D of about 6.5 is demanded, a value contrary to our data.

Quite plainly the open ion postulate leads to a conclusion contrary to the experimental facts. In Table I it may be seen that in both methoxyalcohol systems *the terms k_{-2}/k_3 are nearly insensitive to isotopic substitution.* The clear implication is that there must be a substantial isotope effect in the ion-forming process (step 2). Thus, our original contention of hydrogen participation is confirmed. Since the rearrangement occurs through an internal hydrogen migration, it is logical to conclude that the ionic intermediate is best written as a hydrogen-bridged ion of the type



The relative magnitudes of the values for the k_{-2}/k_3 terms in the systems III–IV and VII–VIII are also consistent with the postulate of a nonclassical intermediate. Bearing in mind that the statistical factor of two migrating hydrogens or deuteriums in III–IV must be included in any comparison of these systems, it is seen that the process of hydrogen or deuterium migration is favored over reaction with water by nearly a factor of four in VII–VIII as compared to III–IV. This clearly reflects the driving force for hydrogen migration provided by the substitution of a methyl group on the β-carbon.

Acknowledgment.—The authors wish to acknowledge gratefully the support of this work by the U. S. Atomic Energy Commission under Contract No. AT (11-1)-555. We also wish to thank Mr. Hans-Georg Gilde for preparing some of the compounds used in this study.

(9) K. B. Wiberg, *Chem. Revs.*, **55**, 713 (1955).

(10) C. J. Collins, W. T. Rainey, W. B. Smith and L. A. Kaye, *J. Am. Chem. Soc.*, **81**, 460 (1959).

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, UNIVERSITY OF MINNESOTA, MINNEAPOLIS, MINNESOTA]

The Biogenesis of the Alkaloids of *Colchicum*. II. Tracer Studies with Acetate-1-C^{14} and Methionine-methyl-C^{14}[1]

BY EDWARD LEETE AND PIROSKA E. NÉMETH

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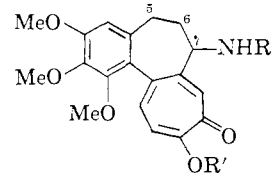
The administration of sodium acetate-1-C^{14} to *Colchicum byzantinum* corms led to the formation of radioactive colchicine which was labeled on the N-acetyl group, negligible activity being found in the rest of the molecule. Demecolcine isolated from the same plants was essentially inactive. Radioactive colchicine and demecolcine labeled on their O- and N-methyl groups were obtained when L-methionine-methyl-C^{14} was fed to the *Colchicum* plants.

In our first paper on the biogenesis of colchicine[2] we found that the administration of phenylalanine-3-C^{14} to *C. byzantinum* corms led to the formation of colchicine (I) labeled at C-5. There was no activity in the rest of the molecule, and we speculated that the tropolone ring was formed in part from acetic acid especially since it has been shown that six of the carbons of the tropolone ring of the mold metabolite puberulic acid are derived from acetic acid.[3]



I R = COMe, R′ = Me
II R = R′ = Me
III R = R′ = H
IV R = COMe, R′ = H
V R = Me, R′ = H

(1) This work was presented in part at the I.U.P.A.C. symposium on the chemistry of natural products, Australia, August, 1960. This investigation was suported by a research grant MY-2662, from the National Institute of Mental Health, Public Health Service.

(2) E. Leete and P. E. Németh, *J. Am. Chem. Soc.*, **82**, 6055 (1960).

We therefore administered sodium acetate-1-C^{14} to flowering *C. byzantinum* corms by the method previously described.[2] Colchicine was isolated from the plants four weeks later and was found to be radioactive. Hydrolysis of the alkaloid with dilute sulfuric acid[4] yielded trimethylcolchicinic acid (III) which had negligible activity and acetic acid which was isolated as its sodium salt. This sodium acetate had the same specific activity as the colchicine indicating that the acetate fed was serving only as a precursor of the N-acetyl group of colchicine. Demecolcine (II) was also isolated from the corms which had been fed radioactive acetate. This alkaloid, which lacks an N-acetyl group, had negligible activity. Our results are in agreement with the independent experiments of Battersby and Reynolds[5] who fed sodium acetate-1-C^{14} to *C. autumnale* plants and obtained radioactive colchicine labeled almost entirely on the N-acetyl group. It thus seemed that acetate was not a precursor of the tropolone ring of colchicine and demecolcine. However negative results of this type should be interpreted with caution. The tropolone ring of the alkaloids of *Colchicum* could have been formed from acetate prior to the feeding of the radioactive acetate. Then at the time of feeding it is possible that the "preformed" tropolone ring was no longer in metabolic equilibrium with acetate. Further tracer experiments will be necessary to resolve this problem.

In another experiment we fed L-methionine-methyl-C^{14} to flowering *C. byzantinum* corms and, as expected, we obtained radioactive colchicine and demecolcine. Systematic degradation of these alkaloids by established methods indicated that all the activity was located on their peripheral methyl groups and none in their carbon skeletons. In colchicine 10% of the activity was located on the O-methyl of the tropolone ring and 90% was present on the three methoxyl groups of the benzene ring. In demecolcine 26% of the activity was located on the O-methyl group of the tropolone ring, 13% on the N-methyl group and the rest on the methoxyl groups of the benzene ring. At this stage of our study on the biogenesis of the *Colchicum* alkaloids, it is not possible to attach any significance to the different levels of activity of the methyl groups located at different positions in the alkaloid molecules.

Experimental[6]

Administration of Tracers to *Colchicum* Corms and Isolation of the Colchicine and Demecolcine.—Aqueous solutions of sodium acetate-1-C^{14} [7] (14.3 mg., total activity 1.92×10^9 c.p.m.) and L-methionine-methyl-C^{14} [8] (53.7 mg., total activity 5.15×10^7 c.p.m.) were fed to sprouting *C. byzantinum* corms in September 1959 by the method previously described.[2] Ten corms were used in each experiment and they were harvested one month after the initial feeding of the tracers. The colchicine was isolated as previously described.[2] The final step in the purification of the colchicine involved solution in chloroform followed by dilution with petroleum ether (b.p. 40–60°) when colchicine crystallized out. When the supernatant chloroform–petroleum ether layer was evaporated to small bulk and cooled overnight large crystals separated out. This material was crystallized several times from ethyl acetate to yield colorless prisms of demecolcine, m.p. 183–184°. Further purification was achieved by chromatography on alumina (activity III), eluting with benzene and then with a mixture of benzene and chloroform (4:1) when pure demecolcine, m.p. 184–185°, was obtained.

Anal. Calcd. for $C_{21}H_{25}O_5N$: C, 67.90; H, 6.78; N, 3.77. Found: C, 67.92; H, 6.94; N, 3.63.

Its ultraviolet and infrared spectra were identical with those of an authentic specimen of demecolcine (substance F) generously provided by Professor F. Šantavý, Palacký University, Olomouc, Czechoslovakia.

The yields and activities of these alkaolids are recorded in Table I.

TABLE I

	Wt. (mg.)	Activity[9] (c.p.m./mM)	Percentage incorporation
Acetate-1-C^{14} experiment			
Colchicine	336	2.05×10^5	0.01
Demecolcine	333	$<0.02 \times 10^5$	..
L-Methionine-methyl-C^{14} experiment			
Colchicine	326	6.0×10^5	0.95
Demecolcine	344	2.03×10^5	0.37

Degradation of the Radioactive Colchicine Derived from the Acetate-1-C^{14}.[3]—The active colchicine (336 mg.) obtained from the plant was mixed with inactive colchicine and crystallized from ethyl acetate to yield material having a specific activity of 3.7×10^4 c.p.m./mM. A portion of this diluted colchicine (159 mg.) was heated on a steam-bath with a mixture of water (3 ml.) and concentrated sulfuric acid (0.75 ml.) for 5 hr. The solution was then neutralized with sodium carbonate and the pale yellow precipitate filtered off and washed with a little cold water. The precipitate was dissolved in water (15 ml.) and extracted with chloroform. The dried chloroform extract was evaporated and the residue crystallized from ethanol–petroleum ether to yield pale yellow needles of trimethylcolchicinic acid (III) (76 mg.) which after several crystallizations was completely devoid of activity. The aqueous filtrate obtained after removal of the crude III was acidified with sulfuric acid and distilled. The distillate was neutralized with sodium hydroxide and evaporated to dryness to yield sodium acetate (4.2 mg.) which was dried *in vacuo* at 100°. This material had an activity of 3.6×10^4 c.p.m./mM.

Degradation of the Colchicine Derived from the L-Methionine-methyl-C^{14}.—The active colchicine was diluted with about 1 g. of inactive alkaloid and the degradations carried out on this material. Colchiceine (IV), trimethoxyphthalic anhydride and trimethoxybenzoic acid were obtained from the colchicine by previously described methods.[2] Gallic acid was obtained as follows. 3,4,5-Trimethoxyphthalic anhydride (15 mg.) was refluxed with hydriodic acid (1.0 ml., d. 1.5) for 3 hr. The solution was extracted with ether and the extract washed with aqueous sodium thiosulfate solution. The dried ether extract was evaporated to yield gallic acid, m.p. 235–238° (dec.) (5 mg.), having an infrared spectrum identical with authentic material. The specific activities of these colchicine degradation products are recorded in Table II and are calculated for carrier free material.

Degradation of the Demecolcine Derived from the L-Methionine-methyl-C^{14}. (a) Oxidation.—Demecolcine (400 mg.) was oxidized with alkaline potassium ferricyanide using the same conditions as those used to oxidize colchicine[2] yielding 3,4,5-trimethoxyphthalic anhydride (47 mg.).

(3) J. H. Richards and L. D. Ferretti, *Biochem. Biophys., Research Comm.*, **2**, 107 (1960), *Proc. Nat. Acad. Sci.*, **46**, 1438 (1960).

(4) R. F. Raffauf, A. L. Farren and G. E. Ullyot, *J. Am. Chem. Soc.*, **75**, 5292 (1953).

(5) A. R. Battersby and J. J. Reynolds, *Proc. Chem. Soc.*, 346 (1960).

(6) Melting points are corrected. Analyses were carried out by Mrs. Olga Hamerston and her assistants at the University of Minnesota.

(7) Purchased from Research Specialties Co., Berkeley, California.

(8) Prepared from methyl iodide-C^{14} according to the procedure of D. B. Melville, J. R. Rachele and E. B. Keller, *J. Biol. Chem.*, **169**, 419 (1947).

(9) Counts were carried out in a Nuclear-Chicago model D-47 Q gas flow counter using a "Micromil window." Determinations were carried out on samples of finite thickness, making corrections for efficiency and self absorption.

TABLE II

ACTIVITIES OF THE COLCHICINE AND DEMECOLCINE OBTAINED FROM THE L-METHIONINE-METHYL-C^{14} FEEDING EXPERIMENT AND THEIR DEGRADATION PRODUCTS

(C.P.M./m*M*)

	Colchicine	Demecolcine
Free alkaloid	6.0×10^5	2.03×10^5
Colchiceine or demecolceine	5.4×10^5	1.50×10^5
3,4,5-Trimethoxyphthalic anhydride	5.4×10^5	1.23×10^5
3,4,5-Trimethoxybenzoic acid	5.5×10^5	1.22×10^5
Gallic acid	0	0
N-Methylbenzamide	.....	0.26×10^5

Further degradation of this anhydride proceeded as for colchicine.

(b) **Demecolceine (V).**[10] Demecolceine (50 mg.) was refluxed for 1 hr. with 2% hydrochloric acid (4 ml.). The cooled solution was neutralized with sodium bicarbonate and extracted with chloroform. The dried extract was evaporated and the residue crystallized from methanol to yield pale yellow needles of demecolceine (44 mg.), m.p. 137–138°. The analytical sample was dried *in vacuo* at 100°.

Anal. Calcd. for $C_{20}H_{23}O_6N$: C, 67.21; H, 6.49; N, 3.92. Found: C, 67.22; H, 6.43; N, 3.95.

(c) **N-Methylbenzamide.**[11]—Demecolcine (100 mg.) was heated at 170° with concentrated hydrochloric acid (3 ml.) in a sealed tube for 12 hr. The contents of the tube were filtered and the filtrate evaporated to dryness. The residue was made alkaline with aqueous sodium hydroxide and the solution distilled into dilute hydrochloric acid. This acid solution was evaporated to small bulk and treated with a mixture of benzoyl chloride and sodium hydroxide to yield N-methylbenzamide which was extracted with ether. The dried ether extract was evaporated and the residue crystallized from ether–petroleum ether to yield colorless needles (16 mg.), m.p. 78–79°, not depressed on admixture with an authentic specimen of N-methylbenzamide.

Chromatography of the Alkaloids.—Pure benzene was an excellent solvent for the separation of the *Colchicum* alkaloids, producing discrete spots which were detected by their fluorescence in ultraviolet light. R_f values obtained with this solvent on Whatman No. 4 paper were: colchicine 0.58, colchiceine 0.67, demecolcine 0.84 and demecolceine 0.92.

(10) A. Uffer, O. Schindler, F. Šantavý and T. Reichstein, *Helv. Chim. Acta*, **37**, 18 (1954).

(11) F. Šantavý, R. Winkler and T. Reichstein, *ibid.*, **36**, 1319 (1953).

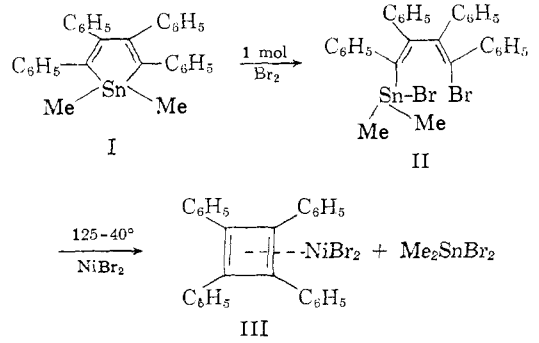
COMMUNICATIONS TO THE EDITOR

TETRAPHENYLCYCLOBUTADIENE DERIVATIVES. I. TETRAPHENYLCYCLOBUTADIENE NICKEL BROMIDE COMPLEX

Sir:

The preparation of authentic derivatives of cyclobutadiene has been and continues to be the object of many investigations.[1] The suggestion that this highly strained molecule may be stabilized by combination with a transition metal[2] has culminated in the recent preparation of tetramethylcyclobutadiene nickel chloride complex,[3] tetraphenylcyclobutadiene iron tricarbonyl complex,[4] and the silver nitrate complex of unsubstituted cyclobutadiene itself.[5] We now wish to report a novel method for the preparation in high yield of tetraphenylcyclobutadiene nickel bromide complex (III) and some preliminary findings on some of its reactions.

The treatment of 1,1-dimethyl-2,3,4,5-tetraphenylstannole[6] with one mole of bromine in the cold leads to the cleavage of one of the ring carbon–tin bonds and the quantitative production of (4-bromo - 1,2,3,4 - tetraphenyl - *cis,cis* - 1,3 - butadienyl)-dimethyltin bromide (II); m.p. 142–143° (Found: C, 54.32; H, 4.13; Sn, 17.56; Br, 24.19). Proof of structure of II is provided by its further reaction with an additional mole of bromine to give, in 80% yield, *cis,cis*-1,4-dibromo-1,2,3,4-tetraphenylbutadiene, obtained in two crystalline modifications, m.p. 147–148° or 151–152° (Found: C, 65.2; H, 3.5; Br, 31.0). Heating a suspension of one mole of anhydrous nickel bromide in a triglyme solution of II under nitrogen at 130 ± 10° for one hour, furnishes an 80% yield[7] of 1,2,3,4-tetraphenylcyclobutadiene nickel bromide complex (III), as thin, shiny, blue-black plates. Its n.m.r. spectrum in dimethyl sulfoxide shows only phenyl hydrogen and its analysis, after recrystallization from bromobenzene, is correct for $C_{28}H_{20}NiBr_2$ (Found: C, 58.4; H, 3.6; Br, 27.9; Ni, 10.0).



The complex III is thermally stable to approximately 300° at which temperature it decomposes to nickel bromide and as yet unidentified hydrocarbon products. It is stable to air oxidation, but is readily oxidized by sodium nitrite in aqueous dimethylformamide, yielding nickel hydroxide and

(1) For a review of the literature through 1958 see: W. Baker and J. F. W. McOmie in "Non-Benzenoid Aromatic Compounds" (Ed. D. Ginsburg), Interscience Publishers, Inc., New York, N. Y., 1959, pp. 43–105.

(2) H. C. Longuet-Higgins and L. E. Orgel, *J. Chem. Soc.*, 1969 (1956).

(3) R. Criegee and G. Schröder, *Ann.*, **623**, 1 (1959).

(4) W. Hubel, *et al.*, *J. Inorg. Nucl. Chem.*, **9**, 204 (1959).

(5) M. E. Avram, E. Marica and C. D. Nenitzescu, *Ber.*, **92**, 1088 (1959).

(6) F. C. Leavitt, T. A. Manuel, F. Johnson, L. U. Matternas and D. S. Lehman, *J. Am. Chem. Soc.*, **82**, 5099 (1960).

(7) By modifications of the method given in ref. 6 we have been able to prepare the stannole I in yields of better than 80%; the overall yield of the complex III from diphenylacetylene is, therefore, 65%.