sumed sp^2-hybridization within nearly planar ethylene-like residues. The angle between the π-system about atom C_1 and its adjoining ring is 50.3°, and that between C_2 and its adjoining ring is 46.2°. These angles would permit some conjugation, but the double bond distance of 1.35 Å. and the single bond distance of 1.51 Å. within the COT ring as well as the normal sp^2 to sp^2 bond length of 1.50 Å. for both C_1C_3 and C_2C_9 give no indication of abnormal bond shortenings. Indeed, the 1.334- and 1.462-Å. distances of the electron diffraction study[9] and the 1.31- and 1.46-Å. distances of the X-ray diffraction study[10] of COT itself suggest that perhaps intramolecular strain is more important than conjugation in C_8Ph_8. Certainly the ring has not assumed one of the highly distorted forms found[11] in the iron tricarbonyl complexes.

Acknowledgment.—We wish to thank the Office of Naval Research, the National Institutes of Health, and the Advanced Research Projects Agency for support. We are also indebted to P. D. Bartlett for valuable discussions, to D. R. Petersen for the study of possible transformation by X-rays, and to T. R. R. McDonald, W. S. McDonald, and J. I. Jones for communicating to us their results on the $(PhAlNPh)_4$ structure.

(9) O. Bastiensen, L. Hedberg, and K. Hedberg, *J. Chem. Phys.*, **27**, 1311 (1957).

(10) J. Bregman, private communication, 1958.

(11) B. Dickens and W. N. Lipscomb, *J. Chem. Phys.*, **37**, 2084 (1962).

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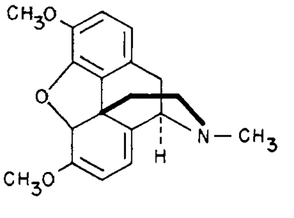
RECEIVED SEPTEMBER 21, 1964

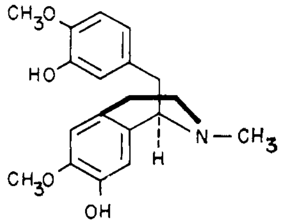
Reticuline as the Benzyltetrahydroisoquinoline Precursor of Thebaine in Biosynthesis with Carbon-14 Dioxide[1]

Sir:

Earlier work[2] established the rapid *de novo* synthesis of thebaine (I) from $^{14}CO_2$ and its primacy in the hydrophenanthrene alkaloid series. Recent evidence, based on feeding experiments, showed that reticuline (II) can serve as a precursor of thebaine.[3] We now present evidence, derived from short exposures to $^{14}CO_2$ of both seedlings and budding plants of *P. somniferum* L., which confirms these findings and establishes beyond question the role of reticuline as the true biosynthetic benzyltetrahydroisoquinoline precursor of the hydrophenanthrene alkaloids.



I



II

If reticuline is a true precursor of thebaine, it should be present with specific activity equal to or greater than that of thebaine after short-term exposure of plants to $^{14}CO_2$. The maximum ratio of the specific activities should exceed the inverse mole ratios of the two alkaloids in the plant, this difference becoming greater with shorter exposure times. To obtain such data, we used gas–liquid chromatography combined with continuous combustion and flow counting,[4] carrying out multiple analysis on single mature plants or a few grams of seedlings. The column temperature was programmed from 180–260°, and the total effluent was passed through a triode–argon detector followed by combustion and counting with a flow-through proportional counter. A dual pen recorder simultaneously plotted the output of a counting ratemeter and the ion current from the triode–argon mass detector, giving mass and radioactivity in differential-type curves. The useful mass range was 0.1 to 20 μg., and activities of about 100 c.p.m. were detectable in any one peak.

A mixture of codeine, thebaine, laudanosine, papaverine, cryptopine, and narcotine was completely resolved ($^3/_{16}$ in. × 6 ft. column, nonpolar silicone gum on silanized Celite), as were the four phenolic alkaloids morphine, laudanosoline, reticuline,[5] and laudanine, as their trimethylsilyl ethers[6] (cyano-silicone polymer as liquid phase). As confirmatory analytical adjuncts two-dimensional chromatography on alumina impregnated paper, paper chromatography using two different systems,[7] and thin layer chromatography[8] were employed.

After the natural occurrence of reticuline[9] in very low concentrations in 120-day poppies and 5-day seedlings was shown by g.l.c. (Table I), several hundred 5-day seedlings were exposed to $^{14}CO_2$ for 2.5 hr., and the alkaloids were extracted immediately (after addition of 1 mg. of reticuline carrier). Gas, paper, and thin layer chromatography showed reticuline to account for more than 80% of the activity in the phenolic alkaloids, while thebaine accounted for more than 50% of the nonphenolic alkaloid activity. Similar results were obtained using a 120-day plant (1 day prior to blossom). Comparison of the specific activities of reticuline and thebaine shows reticuline to have a much higher specific activity than thebaine; the reticuline:

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(1) Sponsored in part by the United States Atomic Energy Commission and Grant B-570 from the National Institutes of Health, United States Public Health Service.

(2) F. R. Stermitz and H. Rapoport, *J. Am. Chem. Soc.*, **83**, 4045 (1961).

(3) A. R. Battersby, R. Binks, D. M. Foulkes, R. J. Francis, D. J. McCaldin, and H. Ramuz, *Proc. Chem. Soc.*, 203 (1963); D. H. R. Barton, G. W. Kirby, W. Steglich, and G. M. Thomas, *ibid.*, 203 (1963).

(4) F. Cacace, A. Guarine, and Inam-ul-Haq, *Ann. Chim.* (Rome), **50**, 915 (1960).

(5) K. W. Gopinath, T. R. Govindachari, and N. Viswanathan, *Ber.*, **92**, 1657 (1959).

(6) Prepared according to C. C. Sweeley, R. Bentley, M. Makita, and W. W. Wells, *J. Am. Chem. Soc.*, **85**, 2497 (1963).

(7) M. Tomita and I. Kikkawa, *Chem. Pharm. Bull.* (Tokyo), **4**, 230 (1956); T. Swain, *Biochem. J.*, **53**, 200 (1953).

(8) D. Walki, K. Schnackerz, and F. Munter, *J. Chromatog.*, **6**, 61 (1961).

(9) The isolation from opium of a compound characterized as *dl*-reticuline was reported by E. Brockmann-Hanssen and T. Furuya at the Third International Symposium on Chemistry of Natural Products, Kyoto, Japan, April 13, 1964. This finding, if correct, raises the question as to the fate of (*S*)-(+)-reticuline, since only the (*R*)-(−) isomer should lead to thebaine (assuming no inversion during the conversion), and since there is no accumulation of reticuline. (*S*)-(+)-Reticuline may prove to be the precursor of narcotine, the major nonphenanthrene alkaloid in this species of poppy, which has been shown[10] to have the same steric configuration as (*S*)-(+)-reticuline. In this connection, it is noteworthy that narcotine appears in the seedlings a few days after the maximum thebaine concentration is found.

(10) M. Ohta, H. Tani, S. Morozume, S. Kodaira, and K. Duritama, *Tetrahedron Letters*, 1857 (1963); A. R. Battersby and H. Spencer, *ibid.*, 11 (1964).

thebaine ratios are 20:1 and 25:1 for 5-day seedlings and the budding plant, respectively. The reticuline: thebaine molar ratios are 1:10 and 1:20 for the two age groups, respectively. Thus, the condition necessary to establish reticuline as the thebaine precursor has been met.

TABLE I

RETICULINE–THEBAINE RELATIONSHIPS IN BIOSYNTHESIS WITH $^{14}CO_2$

Plant age (days)	5	5	120
$^{14}CO_2$ absorbed (mc.)	2.4	3.8	3.0
Duration of exposure (hr.)	2.5	2.75	1.0
Total alkaloid activity (d.p.m./mc./per hr. exposure per g. plant)	7.0×10^4	5.4×10^4	1.5×10^4
Total thebaine d.p.m./ reticuline d.p.m.	4:1	6:1	3:1
Thebaine sp. act. (d.p.m./ μmole)	4×10^5	9×10^4	4×10^5
Reticuline sp. act.[a] (d.p.m./μmole)	8×10^6	5×10^6	1×10^7
Thebaine concn. in plant (μmoles/g.)	0.1	0.1	0.012
Reticuline concn. in plant (μmoles/g.)	0.01	0.01	0.0006
Carrier reticuline added (μmoles)	3	0	2.7
Thebaine/reticuline molar ratios[a]	10:1	10:1	20:1

[a] Calculations are based on the maximum reticuline concentration.

Even if the mass of reticuline present was so low as to escape detection by g.l.c., one would expect to see a radioactivity peak for reticuline of the same magnitude as for thebaine, unless the rate of reticuline turnover is different from that of thebaine, because the reticuline:thebaine specific activity ratio must be at least equal to their inverse mass ratio.[11] Indeed, analysis of the alkaloids from 5-day seedlings exposed to $^{14}CO_2$ as above, but with no carrier added, showed a large activity, but no mass, peak for reticuline. Thebaine showed similar activity but with a substantial mass peak. Since the ratio of the *total* activities of thebaine:reticuline was from 3 to 6:1, and both compounds are known to be recovered to the same extent, it is clear that reticuline is more rapidly turned over than thebaine. This illustrates the potential of the gas chromatographic method with simultaneous mass and activity measurement, used in conjunction with $^{14}CO_2$ feeding of plants, to detect small amounts of active metabolites and to compare specific activities of related compounds.

Further support for the view that all the carbons of reticuline are incorporated into thebaine is given by the fact that the fraction of activity in the O- and N-methyl groups is substantially the same for thebaine (20%) and reticuline (17%) from a 5-day seedling experiment.

The early appearance of high concentrations of thebaine and the high rate of incorporation of radioactivity into the total alkaloid fraction as well as into thebaine and reticuline both in seedlings and in mature plants should place beyond doubt the intimate involvement of these alkaloids in the economy of the plant.[12] Our findings also constitute positive evidence for the operation of the same biosynthetic relationships (*i.e.*, CO_2 → reticuline → thebaine → codeine → morphine) in seedlings as in mature plants.[13,14]

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[11] Assuming the *de novo* synthesis of reticuline and thebaine is small, and an average value of activity is incorporated into both alkaloids during the period of $^{14}CO_2$ exposure.

[12] J. W. Fairbairn and G. Wassel, *Phytochemistry*, **3**, 253 (1964), present additional evidence for active metabolic roles for the morphine-type alkaloids, as suggested by the re-feeding experiments of F. R. Stermitz and H. Rapoport.[2]

[13] J. Massicot, *Ann. Pharm. Franc.*, **19**, 44 (1961), suggested that different metabolic pathways for the morphine-type alkaloids exist in seedlings compared with mature plants.

[14] D. Neubauer, *Planta Med.*, **12**, 43 (1964), presented negative though complementary evidence to support this view.

[15] U. S. Public Health Service Postdoctoral Fellow.

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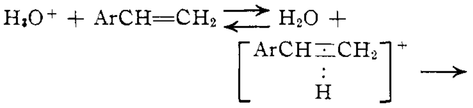
RECEIVED SEPTEMBER 8, 1964

The Hydration of Styrenes

Sir:

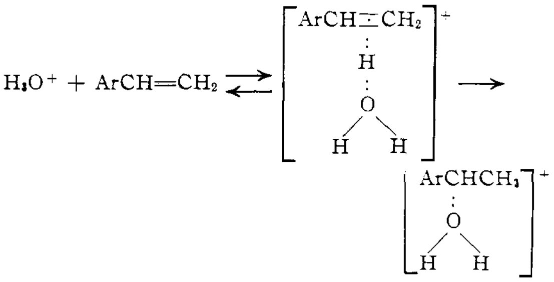
We have obtained evidence consistent with the rate-determining step in the hydration of styrenes being a proton transfer to styrene to form a carbonium ion (mechanism I). Rate-determining rearrangement of an "unencumbered" π-complex between olefin and a proton is excluded (mechanism II).[1]

$$\mathrm{H_3O^+ + ArCH{=}CH_2 \longrightarrow H_2O + ArC^+HCH_3 \longrightarrow products} \quad \mathrm{(I)}$$



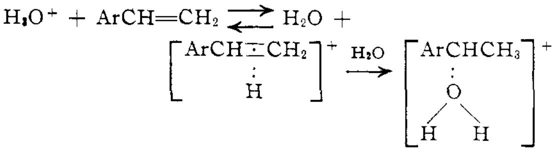
$$\mathrm{H_3O^+ + ArCH{=}CH_2 \rightleftarrows H_2O + \left[\begin{matrix}ArCH\overset{\cdot\cdot}{=}CH_2\\ \vdots\\ H\end{matrix}\right]^+ \longrightarrow products}$$

(II)



$$\mathrm{H_3O^+ + ArCH{=}CH_2 \rightleftarrows \left[\begin{matrix}ArCH\overset{\cdot\cdot}{=}CH_2\\ \vdots\\ H\\ \vdots\\ O\\ H \quad H\end{matrix}\right]^+ \longrightarrow \left[\begin{matrix}ArCHCH_3\\ \vdots\\ O\\ H \quad H\end{matrix}\right]^+}$$

(III)



$$\mathrm{H_3O^+ + ArCH{=}CH_2 \rightleftarrows H_2O + \left[\begin{matrix}ArCH\overset{\cdot\cdot}{=}CH_2\\ \vdots\\ H\end{matrix}\right]^+ \xrightarrow{H_2O} \left[\begin{matrix}ArCHCH_3\\ \vdots\\ O\\ H \quad H\end{matrix}\right]^+}$$

(IV)

The following results have been obtained: (1) Values of the first-order rate constant for hydration, k_{obsd}, and of [Sty]/[ROH] at equilibrium were determined spectrophotometrically in 5–7 M perchloric acid (covering about a 15% range of acid strength) for styrene and four *para*-substituted styrenes. Due to lack of space, only those values of k_{obsd} ($k_{\text{obsd}} = k_{\text{hydr}} + k_{\text{dehydr}}$) and K ($K = k_{\text{dehydr}}/k_{\text{hydr}}$) obtained in the lowest and highest perchloric acid molarities in each case are listed in Table I. The ratio [Sty]/[ROH] = K was small and showed little sensitivity to perchloric acid molarity or temperature. Consequently, errors in

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[1] R. H. Boyd, R. W. Taft, Jr., A. P. Wolf, and D. R. Christman, *J. Am. Chem. Soc.*, **82**, 4729 (1960), and earlier references.