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above for the C-2 labeled compound), and its effect on isotopic equilibration is identical with that of (3).

2-Methyl-2-chlorobutane-1-C^{13} (1.00 g.) was treated with aluminum chloride (0.049 g.) for 5 min. according to ref. 2. The recovered volatile fraction (52%) was shown by v.p.c. to contain 62.8% *t*-amyl chloride, 21.6% *t*-butyl chloride, 7.4% 2-methyl-2-chloropentane, 2.0% 3-methyl-3-chloropentane, 4.1% 2-methyl-3-chlorobutane, 0.9% isopentane and 0.6% methylpentanes. The corresponding reaction of the C-2 labeled compound gave identical results. The *t*-amyl chlorides were collected through a Beckman Megachrom and analyzed with a Consolidated Model 21-103C Mass Spectrometer. The pertinent mass spectrometric data are summarized.

MASS SPECTRAL ANALYSIS OF *t*-AMYL CHLORIDES: ISOTOPIC COMPOSITION (% MOLECULES)

	Before reaction		After reaction	
t-Amyl chloride-1-C^{13}	$C_5H_{11}^+$	$C_5H_{11}^+$	$C_4H_8Cl^{+a}$	$C_3H_6Cl^{+a}$
unlabeled	57.0	61.0	31	30.0
monolabeled	43.0	35.5	66	68.2
dilabeled	0.0	3.5	3	1.8
t-Amyl chloride-2-C^{13}				
unlabeled	42.3	46.9	0.0	47.8
monolabeled	57.7	48.8	94	52.2
dilabeled	0.0	4.3	6	0.0

^a Isotopic composition computed on the basis of labeled molecules only (contribution of unlabeled *t*-amyl chloride removed).

These data, corroborated and supplemented by proton n.m.r., can be summarized

We wish to make several comments: (1) The data constitute cogent arguments for the contribution of *bimolecular paths* to liquid phase isomerizations. (2) Since isotope-position rearrangement has reached equilibrium, reliable quantitative conclusions concerning the per cent. contributions of bimolecular paths (5) and (6), and unimolecular

(3) Values in parentheses are from the C_4 and C_3 fragments. Very little C^{13}, if any, appears in carbons other than those designated.

paths to the over-all rearrangement cannot be drawn. An *upper limit* of 20% contribution by (5), (6), and their analogs to C-1 $\rightleftarrows$ C-4 scrambling, and 14% contribution by (6) and its analogs to C-2 $\rightleftarrows$ C-4 scrambling may be calculated from the data. (3) We have no way of evaluating the contribution of (7), the analog of (3), and in this respect the over-all contribution of bimolecular reactions to isomerization could be higher than the values given above.

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DISTRIBUTION OF RADIOACTIVITY IN MORPHINE FROM BIOSYNTHESIS WITH CARBON-14 DIOXIDE*

Sir:

Three groups of workers[1,2,3] independently fed ^{14}C-labeled tyrosine to plants of *Papaver somniferum* and observed the formation of radioactive opium alkaloids. In two cases[1a,2] the isolated morphine (a hydrophenanthrene alkaloid) and in two cases the isolated papaverine[1b] and narcotoline[3] (benzylisoquinoline alkaloids) were degraded, and in each case the activity was reported as equally divided between the two halves of the molecule, long considered to be derivable from two molecules of tyrosine or an equivalent.[4]

Although the equality of labeling in the two halves was stressed as much as the fact of incorporation, this becomes the "theoretical" distribution only if the alkaloid is formed by the direct combination of two identical molecules or if nonidentical molecules combine and the structure formed or subsequent intermediates become symmetrical, as in the example.

(1) (a) A. R. Battersby and B. J. T. Harper, *Chem. and Ind.*, 364 (1958); (b) A. R. Battersby and B. J. T. Harper, *Proc. Chem. Soc.*, 152 (1959).

(2) E. Lette, *J. Am. Chem. Soc.*, **81**, 3948 (1959).

(3) G. Kleinschmidt and K. Mothes, *Z. Naturforsch.*, **14b**, 52 (1959).

(4) E. Winterstein and G. Trier, "Die Alkaloide," Borntraeger Press, Berlin, 1910, p. 307.
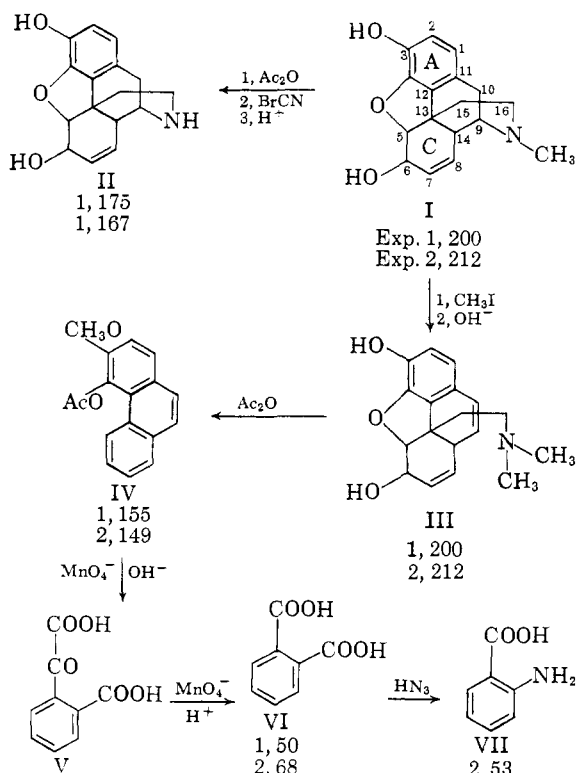
If, however, the structure is formed by combination of non-identical molecules and a symmetrical intermediate does not pertain, then one might expect an activity ratio differing from 1:1 for the two halves since the pool sizes and, hence, specific activities of the various precursors probably would differ considerably. In addition, the low values of incorporation, where values were reported, seemed to leave open the possibility that a pathway through tyrosine might represent only one of several or even an aberrant metabolism.[5]

To obtain evidence on these points, morphine isolated from each of the two separate six-hour $^{14}CO_2$-biosyntheses[8] was diluted and degraded as depicted in the flow sheet to give the various activities shown in d.p.m./μmole of diluted material.

This degradation scheme is similar to that employed by Leete.[2] However, in one important aspect our experience differed from that reported.[2] We could find only traces of phthalic acid from the

oxidation of 4-acetoxy-3-methoxyphenanthrene (IV) by alkaline permanganate. The major product was phthalonic acid (V),[9] identified by direct comparison of the acid and its semicarbazone with authentic samples (m.m.p. and infrared).[10] Acidic permanganate then cleanly oxidized phthalonic to phthalic acid.[11]

In Table I, an exploded view is given of the morphine molecule, divided according to the tyrosine biogenetic hypothesis, along with the activities of the various portions indicated.

TABLE I

LABELING PATTERN IN MORPHINE ISOLATED AFTER SIX-HOUR $^{14}CO_2$-BIOSYNTHESIS

Carbon atoms[a]	Expt. 1 d.p.m./μmole	Expt. 2 d.p.m./μmole
$C_{15,16}$ +[b]	20	18
$C_{9,12}$ O[c] ⎫	50	30
$C_{5,6,7,8,14,13}$ ●[d] ⎭		38
$C_{1,2,3,4,11,10}$ X[e]	105	81
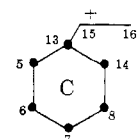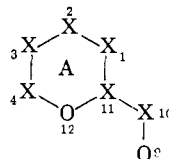
[a] The numbering system of morphine has been retained; ring A corresponds to the aromatic ring, ring C to the cyclohexene ring. [b] This value is the difference between II and IV. [c] Since phthalic acid is symmetrical, this value is 2 (VI–VII). [d] This value is phthalic acid (VI) minus $C_{9,12}$. This value is the difference between IV and VI.

Thus, for Experiment 2, the total activities per biogenetic unit A and C, corresponding to the two postulated tyrosine molecules, are 111 (carbons X and O) and 56 (carbons ● and +), a ratio of 2:1 rather than 1:1 as found in the tyrosine feedings.[1,2,3] In Experiment 1, the conversion of phthalic acid to anthranilic acid failed so that an exact determination of the activities in the two halves was not possible. However, here also it is clear that the activities of units A and C are not equal. For example, if one assumes that all the activity (50) of the phthalic acid is in the carboxylic carbons ($C_{9,12}$), the ratio of activities in unit A (155) to unit C (20) would be 8:1. The opposite assumption, that none of the phthalic acid activity is in the carboxylic carbons leads to a unit A activity of 105, a unit C activity of 70, and a ratio of 3:2. The true ratio must be between these extremes; thus, Experiment 1 corroborates the inequality found in Experiment 2.

From the data we conclude that the hydrophenanthrene ring structure cannot be formed by the combination of two identical molecules, and that a symmetrical intermediate is not involved.[12] The equivalence found in other work[1,2,3] may be

(5) For a further discussion of this point, see F. R. Stermitz and H. Rapoport, *J. Am. Chem. Soc.*, **83**, 4045 (1961). Of interest also are two recent reports[6,7] which show that tyrosine can be incorporated into glucose, in one case[6] in more significant amounts than into berberine, an alkaloid bearing a close structural relationship to some of the opium alkaloids.

(6) I. Imaseki, R. Oneyama and M. Tajima, *Yakugaku Zasshi*, **80**, 1802 (1960).

(7) R. K. Ibrahim, S. G. Lawson and G. H. N. Towers, *Can. J. Biochem. Physiol.*, **39**, 873 (1961).

(8) H. Rapoport, F. R. Stermitz and D. R. Baker, *J. Am. Chem. Soc.*, **82**, 2765 (1960).

(9) From a consideration of the probable path of oxidation through the phenanthrenequinone and the diphenic acid, the keto group of phthalonic acid presumably corresponds to carbon-12 of the morphine. However, this assignment remains to be proved.

(10) A. Cornillot, *Ann. chim. (Paris)*, [10] **7**, 275 (1927).

(11) G. Charrier and A. Beretta, *Gazz. chim. ital.*, **54**, 988 (1924).

(12) In preliminary work on narcotine, a benzylisoquinoline alkaloid, we have found higher activity in the benzyl portion than in the isoquinoline portion.

the result of long term feeding, or tyrosine incorporation may represent only a minor or aberrant biosynthetic pathway. Further data, particularly in regard to the rate of incorporation for individual carbon atoms, are being sought through degradation of thebaine, the primary hydrophenanthrene alkaloid,[5] and should allow proposal of a more definite scheme.

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2-CHLOROCYCLOHEXANONE. ENERGY CALCULATIONS FROM INFRARED ABSORPTION BANDS BY AREA VS. PEAK-INTENSITY MEASUREMENTS

Sir:

As for our estimated value[1] of the energy difference ΔE between the e- and the a-isomer of 2-chlorocyclohexanone, Allinger, et al.,[2] pointed out that their values were somewhat different from ours, especially for the carbon disulfide solution and they described concentration as a probable cause for the discrepancy. We have now investigated concentration effects and conclude that our data are still valid. The approximation method, using peak intensities, gives incorrect results.

As has been pointed out,[2] infrared evidence does show that the energy difference between the two isomers in a non-polar solution decreases when the dilution proceeds. This behavior may be explained in terms of Onsager's reaction field, because the stable e-isomer is more polar than the other. But it was observed that the intensity ratio (C_aA_a/C_eA_e) of the bands assigned to both isomers, respectively, viz., the value of ΔE, becomes constant within experimental error, after the dilution proceeds to some extent. The experimental evidence for this is shown in Table I. Area intensities were determined according to the equation $A = K(1/CL) \ln (T_0/T)\nu_{max} \times \Delta\nu_{1/2}{}^a$, where the letters have their usual meanings. The value of K was obtained from the table of Ramsay.[3] The suffixes a and e refer to both isomers, respectively. The experimental method was the same as described before.[1]

As can be seen from Table I, the ratio of the observed area intensity of the band at 915 cm.$^{-1}$ to that at 932 cm.$^{-1}$, viz. C_aA_a/C_eA_e, is 2.6 in the dilute carbon disulfide solution for the concentration range 0.0496 to 0.199 mole/l. This value agrees with that previously reported by us at 0.37 mole/l. and shows the validity of our earlier results.

The value determined in n-heptane solution does not differ from the above result within experimental error. The change in the solvent from carbon disulfide to n-heptane does not seriously alter the result, since the dielectric constants of both solvents are nearly the same. The data are shown in Table II. As for these cases the results

(1) K. Kozima and Y. Yamanouchi, J. Am. Chem. Soc., **81**, 4159 (1959).

(2) N. L. Allinger, J. Allinger, L. A. Freiberg, R. F. Czaja and N. A. Lebel, ibid., **82**, 5876 (1960).

(3) D. A. Ramsay, ibid., **74**, 72 (1952).

TABLE I
DEPENDENCE OF THE OBSERVED INTENSITY RATIO ON CONCENTRATION

Temp., 21°; solvent, CS_2; spectral slit-width, $S = 2.78$ cm.$^{-1}$ at 915 cm.$^{-1}$, $S = 2.87$ cm.$^{-1}$ at 932 cm.$^{-1}$

C (mole/l.)	ν_{max}	Form	$\ln (T_0/T)\nu_{max}$	$\Delta\nu_{1/2}{}^a$	K	C_aA_a/C_eA_e
0.794	915	a	0.745	5.2	1.49	2.1
	932	e	.213	8.7	1.51	
.397	915	a	.677	5.4	1.48	2.3
	932	e	.181	8.7	1.51	
.199	915	a	.896	5.4	1.50	2.6
	932	e	.212	8.7	1.51	
.0993	915	a	.970	5.2	1.52	2.6
	932	e	.241	8.1	1.51	
.0496	915	a	1.39	5.4	1.56	2.6
	932	e	.315	9.5	1.53	

TABLE II
ENERGY DIFFERENCE BETWEEN TWO ISOMERS BY USE OF THE BANDS AT 915 AND 932 CM.$^{-1}$

Prism, NaCl; spectral slit-width, $S = 2.78$ cm.$^{-1}$ at 915 cm.$^{-1}$, $S = 2.87$ cm.$^{-1}$ at 932 cm.$^{-1}$.

Temp	ν_{max}	Form	$\ln (T_0/T)\nu_{max}$	$\Delta\nu_{1/2}{}^a$	K	C_aA_a/C_eA_e	Ratio of peak intensities[a]
(1) CS_2 solution (0.37 mole/l.)							
24°	915	a	1.00	4.8	1.53	2.6	4.4
	932	e	0.232	8.0	1.54		
−24°	915	a	1.18	3.7	1.54	2.0	3.5
	932	e	0.346	6.4	1.52		

ΔE (by area intensity) 0.81 kcal./mole (stable form, e)
ΔE (by peak intensity) 0.73 kcal./mole

(2) n-Heptane solution (0.82 mole/l.)							
56°	915	a	1.30	5.8	1.56	4.5	5.8
	932	e	0.226	7.7	1.49		
7°	915	a	1.62	4.7	1.59	3.5	4.5
	932	e	0.359	6.4	1.48		

ΔE (by area intensity) 0.92 kcal./mole (stable form, e)
ΔE (by peak intensity) 0.94 kcal./mole

TABLE III
ENERGY DIFFERENCE BETWEEN TWO ISOMERS BY USE OF THE BANDS AT 445 AND 478 CM.$^{-1}$

Prism, KBr; spectral slit-width, $S = 3.24$ cm.$^{-1}$ at 445 cm.$^{-1}$, $S = 3.71$ cm.$^{-1}$ at 478 cm.$^{-1}$.

Temp.	ν_{max}	Form	$\ln (T_0/T)\nu_{max}$	$\Delta\nu_{1/2}{}^a$	K	C_aA_a/C_eA_e	Ratio of peak intensities
(1) CS_2 solution (0.086 mole/l.)							
27°	445	a	0.928	7.4	1.52	0.87	0.83
	478	e	1.12	7.0	1.54		
−20°	445	a	1.40	4.8	1.56	0.71	0.95
	478	e	1.48	6.4	1.56		

ΔE (by area intensity) 0.64 kcal./mole (stable form, e)
ΔE (by peak intensity) −0.40 kcal./mole

(2) n-Heptane solution (0.097 mole/l.)							
41°	445	a	0.988	10.6	1.55	1.13	0.94
	478	e	1.06	8.8	1.54		
−15°	445	a	1.35	5.9	1.56	0.87	0.96
	478	e	1.40	6.5	1.56		

ΔE (by area intensity) 0.75 kcal./mole (stable form, e)
ΔE (by peak intensity) −0.10 kcal./mole

did not change significantly even when the peak intensities[2] were used for the evaluation. How-