Anal. Caled. for $C_{17}H_{17}NO_4;\ C,\ 68.25;\ H,\ 5.75;\ N,\ 4.69.$ Found: C, $68.34;\ H,\ 5.80;\ N,\ 4.69.$

(-)-2-(N-Carbobenzoxyanilino)propionpiperidide (XII).---A cooled solution of 4 g. (0.013 mole) of X in 10 ml. of methanol was titrated with 1 M methanolic NaOH or NaOMe to a phenolphthalein end point. The methanol was removed and replaced with a mixture of ether-Skellysolve B. After the material was collected and dried at 65° (0.1 mm.) for 36 hr., there was obtained 3.16 g. of sodium salt. This salt, partially soluble in ether, was placed in 20 ml. of ether and cooled to about 0° . To the stirred mixture was added dropwise, 1.4 g. (0.011 mole) of oxalyl chloride in 10 ml. of ether. After all the gas had evolved (15 min.), 1.87 g. (0.02 mole) of piperidine was added to the cooled, stirred mixture over a 15-min, period. The mixture was allowed to stand for 0.5 hr. and filtered. The ether solution was washed with 1 M HCl, then with 1 M NaHCO₃, dried over Drierite, and the ether was removed in vacuo. There was obtained 2.41 g. of an oil which was chromatographed on a silicic acid-chloroform column. The first fraction (0.42 g.) consisted mainly of XVI, an oil; λ_{max} 5.40, 5.60 (anhydride), and 5.86 μ (carbamate). The second fraction (1.54 g.), which contained the desired product, was recrystallized several times from ethyl acetate-Skellysolve B to afford 0.50 g. of XII, m.p. 74–76°, $[\alpha]^{23}D = -60^{\circ} (2C_{c} \text{ in ethanol}),$ λ_{max} 5.88 (carbamate) and 6.03 μ (amide).

Anal. Caled. for $C_{22}H_{26}N_2O_3$: C, 72.21; H, 7.15; N, 7.65. Found: C, 71.96; H, 7.07; N, 7.32.

The infrared spectrum of the above compound was identical with that of racemic XII, m.p. $93-95^{\circ}$, prepared by treating 2.32 g. (0.01 mole) of racemic XIII with 2 g. (0.022 mole) of benzyl chlorformate in a mixture of 15 ml. each of 1.5 *M* NaHCO₃ and CHCl₃. The mixture was stirred vigorously for 6 hr. and the chloroform layer was separated from the aqueous phase. After washing the organic phase with 1 *M* HCl, it was dried over Drierite and the solvent was removed. The oil (2.24 g.) solidified on standing and was recrystallized from ethyl acetate–Skellysolve B.

(-)-2-Anilinopropionpiperidide (XIII).—Intermediate XII (0.47 g., 0.001 mole) in 10 ml. of methanol was shaken for 17 min, with 0.1 g. of palladium on carbon under hydrogen at 1.76

kg./cm.² (25 p.s.i.). The mixture was filtered and the catalyst was washed with methanol. The solvent was removed *in vacuo* to afford 0.26 g, of XIII, m.p. 63–65°, $[\alpha]^{23}D - 13^{\circ}$ (1° in ethanol). The infrared spectrum of XIII was identical with that of the racemic compound.¹

(-)-N¹-Pentamethylene-N²-phenyl-1,2-propanediamine Dipicrate. To a stirred mixture of 0.082 g. (0.002 mole) of LiAlH₄ in 2 ml. of tetrahydrofuran was dropped 0.25 g. (0.001 mole) of NIII in 2 ml. of tetrahydrofuran. The mixture was refluxed for 5 hr., then treated successively with 0.1 ml. of water, 0.2 ml. of $15^{C_{\ell}}$ NaOH, and 0.1 ml. of water, and filtered. The solvent was removed *in cacuo* and the residue was dissolved in 1 ml. of ethanol. Enough saturated ethanolic picric acid was added to produce complete precipitation. The yield of XIV dipicrate, m.p. 132.5-134.5°, was 0.56 g. Recrystallization from ethanol afforded 0.40 g. of the salt, m.p. 134–135.5°, $[\alpha]^{23}D = 60^{\circ} (2^{C_{\ell}})$ in acetone).

Anal. Caled, for $C_{26}H_{28}N_8O_{14}$; C, 46.25; H, 4.23; N, 16.61. Found: C, 46.31; H, 4.41; N, 16.51.

Hydrolysis of (–)-**Phenampromide** (**IV**).—A mixture of 3 ml. of concentrated H₂SO₄, 3 ml. of water, and 1.37 g. (0.005 mole) of (–)-phenampromide³ was heated on a steam bath for 15 hr. The solution was made basic with 4 N NaOH and extracted with ether. The organic phase was dried, and the solvent was removed *in vacuo*. To the residue was added 4 ml. of ethanol and enough saturated alcoholic pieric acid to ensure complete precipitation. The yield of XIV dipicrate, m.p. 133.5–135.5°, was 1.74 g. Recrystallization from ethanol afforded 1.48 g., m.p. 134–136°, $\{\alpha\}^{3}$ b -53° (2° i mactone), of XIV dipicrate. No melting point depression was observed when the salt was mixed with the dipicrate of the dimine XIV obtained from IXa. The infrared (CHCl₃) spectra of the dipicrates obtained by different routes were identical.

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Substituent Constants for Aliphatic Functions Obtained from Partition Coefficients

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From the partition coefficients between 1-octanol and water of a variety of derivatives of the type $C_6H_{5-}(CH_2)_nX$, the partition constants (π) for the aliphatic functions X have been determined. The practical value of the additive character of π for the correlation of biological activity with chemical structure is illustrated with data on the narcotic action of alcohols, esters, ketones, and ether on tadpoles. The relation of π to ΔR_M (a chromatographically determined substituent constant) is shown.

Recently we have shown that substituent constants can be useful in the quantitative correlation of biological activity with chemical structure.³ In particular, we have found that using electronic parameters such as the Hammett σ -constant, p K_a values, or electron densities obtained from molecular orbital calculations with a substituent constant π ($\pi = \log P_X - \log P_H$) obtained from partition coefficients, mathematical expressions could be found for correlation in a wide variety of structure-activity problems. π is a free-energyrelated constant for a functional group and is similar to σ . For example, π for the CH₃ group is found by subtracting the logarithm of the partition coefficient for benzene $(P_{\rm H})$ from that of toluene $(P_{\rm X})$. We have used 1-octanol-water for the solvent system. In evaluating π from partition coefficients obtained with over 200 aromatic compounds, we have observed that π for a given function remains approximately constant in much the same fashion as does σ as long as no strong group interactions occur.⁴

We now report values for functional groups not attached to an aromatic nucleus. These too appear to be approximately constant when strong group interactions are absent.

Table I gives the logarithm of the partition coefficients for a variety of compounds of the type $C_6H_6(CH_2)_nX$. The phenyl group was included for analytical con-(4) T. Fujita, J. Iwasa, and C. Hansch, J. Am. Chem. Soc., **86**, 5175 (1964).

⁽¹⁾ On leave from Okayama University, Okayama, Japan.

⁽²⁾ On leave from Kyoto University, Kyoto, Japan.

^{(3) (}a) C. Hansch and T. Fujita, J. Am. Chem. Soc., 86, 1616 (1964);
(b) C. Hansch and A. R. Steward, J. Med. Chem., 7, 691 (1964);
(c) C. Hansch, A. R. Steward, and J. Iwasa. unpublished results.

LOGARITHM OF THE OCTANOL- WATER PARTITION COEFFICIENTS				
x	C6H6(CH2)3X	C6H5(CH2)2X	C6H6CH2X	C: H3X
Н	3.68 ± 0.01^{a}	3.15 ± 0.01^{a}	2.69 ± 0.01^{a}	2.13 ± 0.01^{a}
F	2.95 ± 0.02			2.27 ± 0.01
Cl	3.55 ± 0.02	2.95 ± 0.01		2.84 ± 0.02
Br	3.72 ± 0.01	3.09 ± 0.02		2.99 ± 0.01
OH	1.88 ± 0.01	1.36 ± 0.01	1.10 ± 0.02	1.46 ± 0.01
$\rm NH_2$	1.83 ± 0.02	1.41 ± 0.01	1.09 ± 0.02	0.90 ± 0.01
COOCH3	2.77 ± 0.01	2.32 ± 0.01	1.83 ± 0.02	2.12 ± 0.02
COOH ^b	2.42 ± 0.01	1.84 ± 0.01	1.41 ± 0.01	1.85 ± 0.01
CN	2.21 ± 0.01	1.72 ± 0.02	1.56 ± 0.02	1.56 ± 0.01
COCH3	2.42 ± 0.01		$1,44 \pm 0.03$	1.58 ± 0.01
$CONH_2$	1.41 ± 0.01	0.91 ± 0.01	0.45 ± 0.03	0.64 ± 0.01
CH(+NH ₃)COO-	-0.36 ± 0.02			
OCOCH3	2.77 ± 0.02	2.30 ± 0.02	1.96 ± 0.01	1.49 ± 0.01
OCH ₃	2.70 ± 0.01			2.11 ± 0.01
$N(CH_3)_2$	2.73 ± 0.01			2.31 ± 0.01
N(CH ₃) ₃ +I-	-2.02°			

		Table I		
LOGAR	ITHM OF THE OCTANC	L-WATER PA	ARTITION (Coefficients

^a Standard deviation. ^b The values for the COOH which varies with the concentration were estimated as previously described.⁴ ^c P for this substance varied considerably, depending on the concentration in the octanol phase. The value reported here is for a concentration of $2.62 \times 10^{-5} M$ in the octanol phase. When this concentration increased to $20.6 \times 10^{-5} M$, log P was -157.

TABLE II

VALUES OF π FOR ALIPHATIC FUNCTIONS				
x	$\log P_{C_6H_5(CH_2)_8X} - \log P_{C_6H_5CH_2CH_2CH_3}$	$\frac{\log P_{C_6H_5}(CH_2)_{3X}}{\log P_{C_6H_5}CH_2CH_3}$	$\log P_{C_6H_5CH_2X} - \log P_{C_6H_5CH_3}$	$\frac{\log P_{C_{\delta}H_{\delta}X}}{\log P_{C_{\delta}H_{\delta}}} -$
F	-0.73 ± 0.03			0.14 ± 0.02
Cl	-0.13 ± 0.03	-0.20 ± 0.02		0.71 ± 0.03
Br	0.04 ± 0.02	-0.06 ± 0.03		0.86 ± 0.02
OH	-1.80 ± 0.02	-1.81 ± 0.02	-1.59 ± 0.03	-0.67 ± 0.02
$\rm NH_2$	-1.85 ± 0.03	-1.74 ± 0.02	-1.60 ± 0.03	-1.23 ± 0.02
COOCH3	-0.91 ± 0.02	-0.83 ± 0.01	-0.86 ± 0.03	-0.01 ± 0.03
COOHª	-1.26 ± 0.02	-1.31 ± 0.02	-1.28 ± 0.02	-0.28 ± 0.02
CN	-1.47 ± 0.02	-1.43 ± 0.03	-1.13 ± 0.03	-0.57 ± 0.02
COCH_3	-1.26 ± 0.02		-1.25 ± 0.04	-0.55 ± 0.02
$CONH_2$	-2.28 ± 0.02	-2.24 ± 0.02	-2.24 ± 0.04	-1.49 ± 0.02
CH(⁺ NH ₈)COO [−]	-4.04 ± 0.03			
OCOCH3	-0.91 ± 0.02	-0.85 ± 0.03	-0.73 ± 0.01	-0.64 ± 0.02
OCH ₃	-0.98 ± 0.02			-0.02 ± 0.02
$N(CH_3)_2$	-0.95 ± 0.02			0.18 ± 0.02
$N(CH_3)_3 + I -$	-5.70^{a}			

^a See Table I.

venience for the spectrophotometric determination of the concentrations of compounds in the partitioned phases. It has the advantage over other ultravioletabsorbing groups such as the carbonyl in that it is rather inert chemically and thus does not interact strongly with the functional groups whose π -values were being determined. Table II gives the "aliphatic" π -values for 14 functional groups. Also included in Tables I and II for purposes of comparison are the corresponding "aromatic" π -values. As one would expect, it is clear from Table II that π for a particular function changes considerably when resonance between the function and the aromatic ring is broken by a CH₂ group. Insulation of the resonance effect permits greater localization of the electrons and, therefore, greater relative solubility in the more polar aqueous phase where the greater possibility for hydrogen bonding occurs. As the aromatic ring is further removed from the function, π appears to become constant. The values for π when n is equal to 2 and 3 are, in most cases, constant within the limits set by experimental error.

Since π appears to be an additive constant as long as strong group interactions are absent, the values in Tables I and II allow one to estimate many log P values. For example, neglecting a very small contribution from log $P_{\rm H}$, the value for methyl alcohol can be obtained as follows.

$$\log P_{C_{6}H_{5}(CH_{2})_{5}OH} - \log P_{C_{6}H_{5}CH_{2}CH_{3}} = \log P_{CH_{5}OH} = -1.27 (1)$$

$$\log P_{C_{6}H_{5}(CH_{2})_{2}OH} - \log P_{C_{6}H_{5}CH_{3}} = \log P_{CH_{5}OH} = -1.33 (2)$$

$$\log P_{C_{6}H_{5}CH_{.}OH} - \log P_{C_{6}H_{5}} = \log P_{CH_{5}OH} = -1.03 (3)$$

Comparison of eq. 1, 2, and 3 again indicates that if the functional groups are removed by 2 or more CH_2 groups, group interaction is small and approximately constant values for log P can be additively determined.

The value for ethyl methyl ketone can likewise be calculated (eq. 4). The experimentally determined value is 0.32.

 $\log P_{C_{6}H_{\delta}(CH_{2})_{3}COCH_{3}} - \log P_{C_{6}H_{5}CH_{3}} = \log P_{C_{2}H_{5}COCH_{3}} = 0.27 \quad (4)$

From this work and that previously reported, a good general purpose value for the methyl or methylene group is 0.52. Thus, adding 1.04 to 0.32 gives 1.36 as the calculated value for butyl methyl ketone. The experimental value is 1.38. Such additivity does not hold, however, when new group interactions not possible in the parts occur in a molecule. For example,

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 $\log P$ for CH₃COCH₂CH₂COOCH₃ was found to be -0.23. Taking the value of 0.32 for ethyl methyl ketone and adding to this -0.91 for the COOCH₃ function gives a calculated value of -0.59. Apparently the polarizing effects of the two strongly electron-attracting functions interact to cause lower water solubility than simple addition would lead one to expect. Previous results^{3a} indicate that electron withdrawal (in this case via an inductive effect) increases π for hydrogen-bonding functions, presumably by tighter binding of lone pair or π -electrons. This reduces the affinity of the function for the aqueous phase thus increasing log P or π . In the above keto ester it is possible that internal hydrogen bonding might also play a subsidiary role. As the groups are further separated, the interactions fall off and the calculated and experimental values come closer into agreement. The experimental value for $CH_3CO(CH_2)_4COOCH_3$ is 0.55. Using the value for butyl methyl ketone and -0.91 for $COOCH_3$ yields a calculated value of 0.47.

A good example of the practical side of the additive nature of log P for the correlation of chemical structure with biological activity comes from the work of Overton on the narcosis of tadpoles by alcohols, esters, ketones, and ether. The log (1/C) values for the isonarcotic concentration are taken from the report of McGowan.⁵ These and the calculated values of log P (Table III) were fitted to the equation log $(1 \ C) = k \log P + c$ by the method of least squares to produce eq. 5. For

$$\log\left(1/C\right) = 0.869 \log P + 1.242 \qquad \begin{array}{c} n & r \\ 28 & 0.965 & 0.229 \end{array}$$
(5)

eq. 5, n is the number of points used in determining the constants, r is the coefficient of correlation, and s is the standard deviation. The correlation obtained with eq. 5 is quite good considering the problem of determining the isonarcotic concentration with tadpoles.

In calculating log P for a chain branching of the type $(CH_3)_2CH_-$, -0.13 was subtracted from log P for the normal chain and for a tertiary grouping, *e.g.*, $(CH_3)_3C_-$, and -0.22 was subtracted from log P for the straight chain. These values were obtained from log P for the corresponding phenoxyacetic acids.⁴

Correlations similar to that obtained by eq. 5 have been obtained using parachor⁶ instead of log *P*. More recently, McGowan⁷ has shown the relationship between π and paracher for a given function. Preliminary comparisons indicate both $\log P$ and parachor give similar results in evaluating the forces³ which limit the concentrations of biologically active molecules reaching the sites of action. Log P or π would appear to be the more useful parameter, partly because of its ease of determination and partly because, through variation of the solvents, one should be able to develop a model system more closely approximating the biophases. In this connection, the work of Green and Marcinkiewicz⁸ is of great importance. Using reversed-phase, tankless, flat-bed chromatography, they have shown that $R_{\rm M}$, which was defined by Bate-Smith and Westall⁹ as $R_{\rm M} = \log [(1/R_{\rm F}) - 1]$, is an additive

(8) (a) J. Green and S. Marcinkiewicz, J. Chromatog., 10, 389 (1963);
(b) S. Marcinkiewicz and J. Green, *ibid.*, 10, 372 (1963).

TABLE III ISONARCOTIC CONCENTRATIONS OF ESTERS, ALCOHOLS, KETONES, AND ETHER WITH TADPOLES

		Log (1/C)	د Log
Compd.	$\log P$	Obsd.	Caled.	(1, C)
CH ₃ OH	-1.270	0.300	0.138	0.162
C_2H_5OH	-0.750	0.500	0.590	0.090
$CH_{3}COCH_{3}$	-0.730	0.650	0.607	0.043
(CH ₃) ₂ CHOH	-0.360	0.900	0.929	0.029
(CH ₃) ₃ COH	0.070	0.900	1.303	0.403
$CH_{3}CH_{2}CH_{2}OH$	-0.230	1.000	1.042	0.042
CH ₃ COOCH ₃	-0.380	1.100	0.911	-0.189
$C_2H_5COCH_3$	-0.270	1.100	1.059	0.041
$HCOOC_2H_5$	-0.380	1.200	0.911	-0.289
$C_2H_5OC_2H_5$	0.590	1.200	1.754	0.554
$(CH_3)_2C(C_2H_5)OH$	0.590	1.200	1.754	0.554
$CH_3(CH_2)_3OH$	0.290	1.400	1.494	0.094
$(CH_3)_2CHCH_2OH$	0.160	1.400	1.381	0.019
$CH_{3}COOC_{2}H_{5}$	0.140	1.500	1.363	0.137
$C_2H_5COC_2H_5$	0.310	1.500	1.511	0.011
$CH_3(CH_2)_4OH$	-0.810	1.600	1.946	0.346
$CH_{3}CH_{2}CH_{2}COCH_{3}$	-0.310	1.700	1.511	-0.189
$CH_3COOCH_2C_2H_5$	0.660	2.000	1.815	-0.185
$C_2H_5COOC_2H_5$	0.660	2.000	1.815	0.185
$(CH_3)_2 CHCOOC_2 H_5$	1.050	2.200	2.154	-0.046
$CH_{3}COOCH_{2}CH(CH_{3})_{2}$	1.050	2.200	2.154	0.046
$CH_3COO(CH_2)_3CH_3$	1.180	2.300	2.267	-0.033
$CH_{3}CH_{2}CH_{2}COOC_{2}H_{5}$	1.180	2.400	2.267	0.133
$CH_3(CH_2)_3COOC_2H_5$	-1.700	2.700	2.719	-0.019
$CH_{3}COO(CH_{2})_{4}CH_{3}$	-1.700	2.700	2.719	-0.019
$C_6H_5COCH_3$	1.580	-3.000	2.615	-0.385
$CH_3(CH_2)_7OH$	2.370	3.400	3.301	-0.099
$\mathrm{CH}_3(\mathrm{CH}_2)_3\mathrm{COO}(\mathrm{CH}_2)_3\mathrm{CH}_3$	2.740	3.600	3.623	0.023

TABLE IV

Correlation of π and $\Delta R_{\rm M}$ of ${\rm XC_6H_4OH}$

			77	π (obsd
х	ΔR_M	Obsd.	Calcd.	caled.)
$4-NH_2$	1.762	-1.63	-1.297	0.333
$3-NH_2$	1.720	-1.29	-1.251	0.039
4-OH	1.450	-0.84	-0.954	0.114
3-OH	1.374	-0.66	-0.869	0.209
4-CN	0.488	0.14	0.108	0.032
3-CN	0.488	0.24	0.108	0.132
$4-CH_3$	-0.165	0.48	0.829	0.349
3-CH₃	-0.165	0.50	0.829	0.329
$4-NO_2$	0.281	0.50	0.337	0.163
$3-NO_2$	0.176	0.54	0.453	0.087
4-Cl	-0.165	0.93	0.829	0.101
3-Cl	-0.165	1.04	0.829	0.211

and constitutive property just as log P is. They have introduced the term $\Delta R_{\rm M}$ for effect of a substituent such as CH₃ upon the $R_{\rm F}$ value of a parent compound. As one would expect, there is indeed a very good linear correlation between π and $\Delta R_{\rm M}$ as is revealed by eq. 6.

$$\pi = -1.103\Delta R_{\rm M} + 0.647 \qquad 0.970 \qquad 0.051 \qquad (6)$$

Equation 6 was derived from the data in Table IV, using π -values⁴ and $\Delta R_{\rm M}$ constants^{8b} obtained from phenols. In measuring $R_{\rm M}$ for the phenols, Trigol and diisopropyl ether were used as the two phases in the tankless chromatography. The excellent correlation obtained with eq. 6 is striking support for eq. 7 suggested by Collander.¹⁰ Collander presented evidence

$$\log P_1 = a \log P_2 + b \tag{7}$$

⁽⁵⁾ J. C. McGowan, J. Appl. Chem., 2, 323 (1952).

⁽⁶⁾ J. C. McGowan, ibid., 4, 41 (1954).

⁽⁷⁾ J. C. McGowan, Nature, 200, 1317 (1963).

⁽⁹⁾ E. C. Bate-Smith and R. G. Westall, Biochem. Biophys. Acta, 4, 427 (1950).

⁽¹⁰⁾ R. Collander, Acta Chem. Scand., 5, 774 (1954).

1 ABLE V	
Log P Values for Miscellaneous	Compounds
Compd.	$\operatorname{Log} P$
$C_6H_5C \equiv CH$	2.53 ± 0.01
$CH_3COC_2H_5$	0.32 ± 0.01
$CH_3CO(CH_2)_3CH_3$	1.38 ± 0.01

$CH_3CO(CH_2)_3CH_3$	1.38 ± 0.01
$CH_3CO(CH_2)_2CH=CH_2$	1.02 ± 0.01
$CH_3COCH_2CH_2 = \triangleleft$	1.50 ± 0.01
Thiophene	1.81 ± 0.01
Indole	1.14 ± 0.01
Pyridine	0.65 ± 0.01
Quinoline	2.03 ± 0.01
$C_6H_5B(OH)_2$	1.58 ± 0.01
$CH_3COCH_2CH_2COOCH_3$	-0.23 ± 0.02
$CH_3CO(CH_2)_4COOCH_3$	0.55 ± 0.03
$ m CH_3(m CH_2)_3 m SCN$	2.03 ± 0.02

that the logarithm of the partition of a compound in one set of solvents (P_1) is linearly related to the logarithm of the partition coefficient in a second similar set of solvents (P_2) .

Since $R_{\rm M}$ and $\Delta R_{\rm M}$ are so readily obtained via tankless chromatography, they should prove to be valuable supplements to log P and π in the extrathermodynamic substituent constant analysis of structure-activity relationships.

Experimental

The partition coefficients were determined according to our previously reported⁴ procedure. Most of the compounds whose log P values are reported in Tables I and V were purified for partitioning by preparative vapor phase chromatography. Several of the compounds employed in this work have not been reported previously.

1-Fluoro-3-phenylpropane.---A mixture of 1-chloro-3-phenylpropane (35 g.), dry powdered potassium fluoride (21 g.), and 120 ml. of ethylene glycol was heated at $150-160^\circ$ for 12 hr. with vigorous stirring. The mixture was then cooled, diluted with water, and extracted with ether. Evaporation of the ether and fractionation of the residue yielded 11 g. of product boiling from 173-195°. This material was purified for partitioning by means of an Aerograph autoprep using a silicon column; b.p. 183.5° $(730.5 \text{ mm.}), n^{23}\text{D} 1.4870.$

Anal. Calcd. for C₉H₁₁F: C, 78.26; H, 7.97. Found: C, 78.01; H, 8.12.

2-Amino-4-phenylpentanoic acid was prepared by the malonic ester method.¹¹ The melting point of our product after recrystallization from water was 245-246° dec.; von Braun and Kruber¹¹ reported 203–206°.

Anal. Calcd. for C₁₁H₁₅NO₂: C, 68.42; H, 7.76. Found: C, 68.47; H, 7.83.

4-Cyclopropyl-2-butanone.--Cyclopropylmethyl bromide was condensed with ethyl acetoacetate in the usual way.¹² The resulting product was hydrolyzed with 5% KOH (yield 33%). The crude material was purified by vapor phase chromatography; b.p. 155° (732 mm.), n^{25} D.14260. Anal. Calcd. for $C_7H_{12}O$: C, 74.94; H, 10.78. Found:

C, 74.89; H, 10.74.

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(11) J. von Braun and O. Kruber, Ber., 45, 389 (1912).

(12) C. S. Marvel and F. D. Hager, "Organic Synthesis," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 248.

The Formation of O-Methylated Catechols by Microsomal Hydroxylation of Phenols and Subsequent Enzymatic Catechol O-Methylation. Substrate Specificity

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A microsomal hydroxylating system which converts phenols to catechols and requires niacinamide adenine dinucleotide phosphate and glucose-6-phosphate has been assayed for a variety of phenols using the enzyme catechol-O-methyltransferase and radioactive S-adenosylmethionine-methyl-C¹⁴. This system specifically methylates catechols converting them to radioactive methoxyphenols which can be extracted and assayed. Among the phenols which are converted to catechols are N-acetylserotonin, hydroxyindoles, tyramine, octopamine, hordenine, metanephrine, morphine, phenazocine, levorphanol, and estradiol. 2,4,6-Trichlorophenol formed an O-methylated product. Products from a variety of substrates were identified by cochromatography with authentic compounds.

Liver microsomes have been shown to hydroxylate a variety of aromatic compounds to phenols.¹⁻³ Recently, Axelrod⁴ has demonstrated that phenolic amines are further hydroxylated by microsomal preparations to yield catechol amines such as (nor)epinephrine and dopamine. The conversion of tyramine to norepinephrine has been demonstrated in vivo,⁵ a finding that

(1) C. Mitoma, H. S. Posner, H. C. Reitz, and S. Udenfriend, Arch. Biochem. Biophys, 61, 431 (1956).

(5) C. R. Creveling, M. Levitt, and S. Udenfriend, Life Sci., 1, 523 (1962).

focuses attention on the group of microsomal hydroxylases which convert phenols to catechols. Among the compounds containing phenolic groups and thus potential substrates for the formation of catechols are various physiologically active hydroxyindoles and phenolic phenethylamines, and a large number of important drugs (morphine, levorphanol, etc.). In order to carry out a survey of substrates, a convenient and widely applicable assay was needed. Catechol-Omethyltransferase⁶ is an enzyme occurring in the soluble supernatant fraction of homogenized liver, which readily

(6) J. Axelrod and R. Tomchick, J. Biol. Chem., 233, 702 (1958).

⁽²⁾ H. S. Posner, C. Mitoma, and S. Udenfriend, ibid., 94, 269 (1961).

⁽³⁾ J. B. Jepson, P. Zaltzman, and S. Udenfriend, Biochim. Biophys. Acta, 62, 91 (1962).

⁽⁴⁾ J. Axelrod, Science, 40, 499 (1963).