thiazolidineacetic acid, formed by quantitative paper chromatography using ninhydrin as the color reagent. From a suitable batch of reaction mixture 6-aminopenicillanic acid (II) has been isolated by absorption on IR-120 (H^+), elution with NH_4OH at *p*H 7.0, concentration *in vacuo*, and adjusting the *p*H to 4.4. The recrystallized product had m.p. 207–208° (dec.) and $[\alpha]D^{25}$ +277 (*C* 1.0 in 0.1 *N* hydrochloric acid).[5] It assayed approximately 2750 u./mg. based on sodium benzylpenicillin by the hydroxylamine colorimetric procedure[6] and by microbiological determination after phenylacetylation[1] (theor., 2752 u./mg.). Acylation of (II) with the appropriate acid chlorides in aqueous acetone buffered at *p*H 7.0 to 7.5 has given good yields of crystalline potassium salts of benzylpenicillin and phenoxymethylpenicillin, which are identical in all respects to the product prepared by fermentation.

Both phenoxymethylpenicillin (V) and allylmercaptomethylpenicillin (O) are hydrolyzed by this microbial acylase system. Details on the distribution of this acylase in microörganisms, and its activity on a series of penicillins, including a large number of new semi-synthetic penicillins will be reported elsewhere.

(5) J. C. Sheehan and K. R. Henery-Logan, THIS JOURNAL, **81**, 5835 (1959), report $[\alpha]^{21}D$ +273 (*C* 1.2 in 0.1 *N* hydrochloric acid).
(6) G. Boxer and P. M. Everett, *Anal. Chem.*, **21**, 670 (1949).

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RECEIVED MAY 28, 1960

SEPARATION OF ALKALOIDS BY GAS CHROMATOGRAPHY

Sir:

In the past, the separation of alkaloids from crude alkaloid mixtures has depended upon fractional crystallization, precipitation, countercurrent extraction and either adsorption or liquid phase partition chromatography. Several recent communications have reported the use of gas phase chromatographic techniques for the separation and identification of steroids[1,2] and high molecular weight fatty primary amines.[3] This communication demonstrates the feasibility of this method for the isolation, separation and identification of alkaloids. Our attention has been focused on alkaloids with molecular weights above 250, since suitable modifications of the conditions should permit separations of lower molecular weight substances without difficulty.[4]

Alkaloids listed in the table gave single component sharp peaks, consistent with the absence of decomposition. A typical sample was 1–3 μl. of a 0.5–1.0% solution of the alkaloid in methanol, acetone or chloroform. In several cases (N-methylcytisine, crinine, ibogaine and solanidine) macro samples were chromatographed and the product was identified as unchanged starting material by standard methods. The power of this analytical tool is illustrated in a separation of *Papaveraceae* alkaloids (Fig. 1).

TABLE I
ALKALOID RETENTION TIMES

Compound	Time, min.[a,b]
1. Lupin alkaloids	
Cytisine	5.1
Methylcytisine	4.3
Methylcytisine N-oxide	5.8
Lupanine	5.5
13-Hydroxylupanine	11.6
Matrine	8.5
Lupinine	1.5[d]
Sparteine	5.9[d]
α-Isosparteine	5.2[d]
13-Hydroxysparteine	14.3[d]
2. Amaryllidaceae	
Galanthine	19.0
Acetylcaranine	10.5
Lycorenine	10.6
Galanthamine	7.8
Crinine	9.5
Powelline	15.8
Tazettine	15.2
Belladine	8.7
3. *Papaveraceae*	
Codeine	8.2
Gnoscopine	90.6
Laudanosine	21.0
Morphine	11.0
Neopine	9.1
Papaverine	35.3
Thebaine	13.2
4. Indole Alkaloids	
Brucine	80.0[c]
Coronaridine	8.2[c]
Ibogamine	15.4
Ibogaine	35.1
Serpentine	16.8[c]
Strychnine	25.9[c]
Voacangine	40.3
5. Steroidal alkaloids	
Solanidine	40.6[c,e]
Solasodine	74.3[c,e]
Tomatidine	77.3[c,e]
6. Miscellaneous	
Atopine	5.0
Caffeine	1.6
Cinchonine	6.7[c]
Cocaine	4.8[c]
Corydaline	16.2[c]
Cryptopine	50.8
Himbacine	12.7[c]
Piperine	33.0
Protopine	44.7
Quinine	11.8[c]

[a] Argon ionization detector, 6 ft. × 4 mm. i.d. columns. [b] Pressure 15 psi., 2–3/100 SE-30 on Chromosorb W, 80–100 mesh, temperature 204° unless otherwise noted. [c] Temperature 222°. [d] Temperature 160°. [e] Pressure 10 psi.

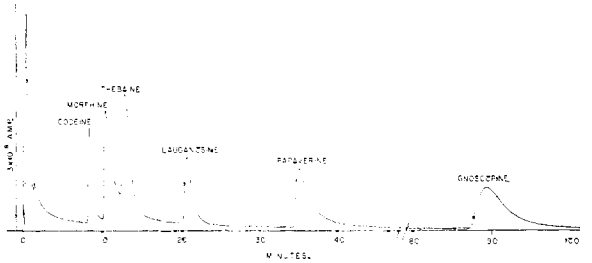

Fig. 1.

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RECEIVED JUNE 16, 1960

THE ENTROPY OF ACTIVATION OF ADDITION OF METHYL RADICALS TO UNSATURATED COMPOUNDS POSSESSING THE SAME REACTION CENTER[1]

Sir:

Addition of methyl radicals to ethylene,[2] propylene,[2] isobutene,[2] styrene,[3] α-methylstyrene,[3] butadiene[4] and isoprene[4] was studied in this

(1) W. J. A. VandenHeuvel, C. C. Sweeley and E. C. Horning, THIS JOURNAL, **82**, 3481 (1960).
(2) R. K. Beerthuis and J. H. Recourt, *Nature*, **186**, 372 (1960).
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(4) *Cf.* L. D. Quin, *J. Org. Chem.*, **24**, 911 (1959).

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(2) R. P. Buckley and M. Szwarc, *Proc. Roy. Soc.*, **A240**, 396 (1957).
(3) F. Leavitt, M. Levy and M. Szwarc, THIS JOURNAL, **77**, 5493 (1955).
(4) A. Rajbenbach and M. Szwarc, *Proc. Roy. Soc.*, **A251**, 1266 (1959).

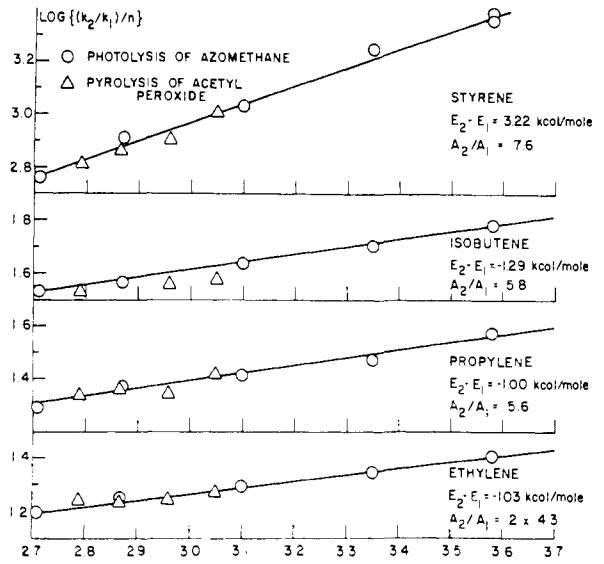

Fig. 1.

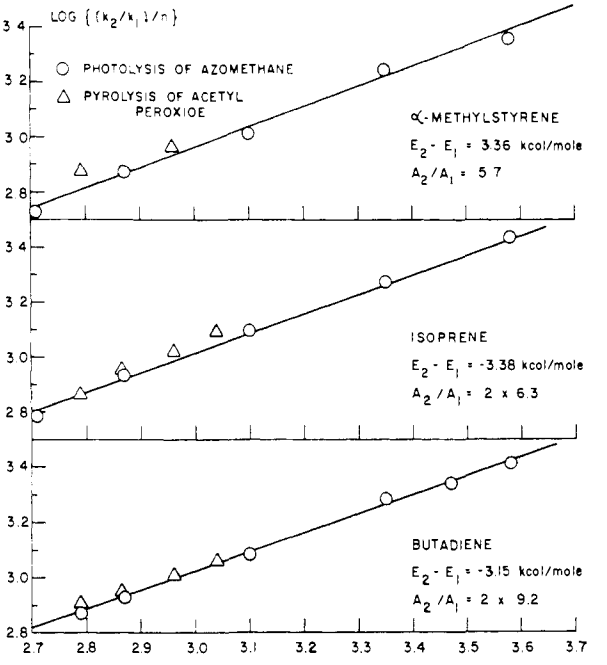

Fig. 2.

laboratory over a temperature range 55–85°, the thermal decomposition of acetyl peroxide being used to generate the radicals. Recently this work was repeated with the certain changes: photolysis of azomethane was used to generate methyl radicals and the temperature range was extended from 6 to 95°. The method applied in the present studies was similar to that developed in previous investigations and the evidence for its justification as well as the details of the experimental technique are given in a paper by Steel and Szwarc.[5]

TABLE I

ACTIVATION ENERGIES AND FREQUENCY FACTORS CALCULATED BY THE LEAST SQUARE METHOD

Compound	k_2/k_1 at 50°[a]	$E_2 - E_1$, kcal./mole	A_2/A_1
Ethylene	39.2	−1.03	2×4.3
Propylene	25.7	−1.00	5.55
Isobutene	42.9	−1.29	5.8
Styrene	1070	−3.22	7.6
α-Methylstyrene	1090	−3.36	5.7
Butadiene-1,3	2440	−3.15	2×9.2
Isoprene	2450	−3.38	2×6.3

[a] The k_2/k_1 values are intrapolated from the respective Arrhenius lines.

In this communication we present only the final results of our studies, shown in Fig. 1 and 2 and Table I, and discuss their significance. The rate constant of methyl radicals addition to the respective substrate is denoted by k_2, whereas k_1 is the rate constant of hydrogen abstraction from isooctane, the latter being used as a solvent in all these experiments. Inspection of Fig. 1 and Table I shows clearly that (1) the same species are responsible for the observed reactions whether acetyl peroxide or azomethane is used to generate the radicals. This means that all these reactions are due indeed to methyl radicals. The suggestion of some workers that acetate radicals are responsible for methylation in the acetal peroxide systems is disproved. (2) The accuracy of activation energies determination is greatly improved in this work as compared with the previous studies. This is obvious from inspection of Fig. 1. Indeed, the present values of $E_2 - E_1$ are reliable within ±0.1–0.2 kcal./mole, whereas the errors in the previous one are ±1–2 kcal./mole. (3) Consequently, the present values of A_2/A_1 are reliable within a factor smaller than 1.3, and inspection of Table I definitely shows that in a series of additions, each involving the same center, A_2/A_1 is nearly constant in spite of a 100-fold change in the reactivities of the investigated substrates. The constancy of A_2/A_1 was assumed in our previous discussions[6,7] but now this assumption is verified experimentally. This shows that the entropy of activation is constant for methyl radical addition reactions to a series of substrates possessing the same type of reaction center.

(5) C. Steel and N. Szwarc, *J. Chem. Phys.*, in press.

(6) M. Szwarc and J. H. Binks, in "Theoretical Organic Chemistry—Kekule Symposium, 1958," Butterworth Publ., 1959, p. 262.

(7) J. H. Binks and M. Szwarc, *J. Chem. Phys.*, **30**, 1494 (1959).

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RECEIVED JUNE 13, 1960

RAUWOLFIA ALKALOIDS. XXXII. THE ABSOLUTE STEREOCHEMISTRY OF AJMALINE AND A NEW PROOF OF ITS STRUCTURE

Sir:

In connection with our current interest in Hunteria alkaloids, we have developed a facile experimental method for the recognition of heterocyclic alcohols related to (III). Ajmaline (I) was used as a source of stereoisomers of this type. We have found that O-tosyl derivatives of the alcohols (III) and (V) may be converted in two steps into N_a-methyl-β-carbolinium salts (VI) which can be recognized through their characteristic ultraviolet