The 3-bicyclo[3.1.0]hexanol[2] with presumably the *cis*-configuration, b.p. 71.0–71.5° (22 mm.), $n^{25}D$ 1.4770, is derived most easily from the action of methylene iodide and zinc–copper couple[3] on Δ^3-cyclopentenol,[4] the yield from this highly stereospecific reaction being 75%. Oxidation of the alcohol or Dieckmann condensation from cyclopropane *cis*-1,2-diacetic acid[5] yields 3-bicyclo[3.1.0]-hexanone, b.p. 54–55° (25 mm.), $n^{25}D$ 1.4590, 2,4-dinitrophenylhydrazone, m.p. 149–150°. Reduction of the bicyclohexanone with lithium aluminum hydride leads to an 89:11 mixture, while aluminum isopropylate gives a 40:60 mixture of the *cis*- and *trans*-alcohols, respectively. Acetolysis of 0.08 *M* *cis*-toluenesulfonate III, m.p. 51.6–51.8°, in the presence of 0.10 *M* sodium acetate at 50° leads exclusively to *cis*-acetate IV with no accompanying olefin. The *trans*-toluenesulfonate, m.p. 70.5–71.5°, gives rise to the same *cis*-acetate with considerable accompanying olefin (*ca.* 33%).

In acetolysis the *cis*-toluenesulfonate III is substantially more reactive than the *trans*-epimer. With the *cis*-toluenesulfonate III the addition of lithium perchlorate in the acetolysis at 50° gives rise to a special salt effect,[6] the magnitude being measured by a (k^0_{ext}/k^0_t) value of 3.2. A special salt effect does not occur in acetolysis of analogous toluenesulfonates, such as Δ^3-cyclopentenyl, cyclopentyl and cyclohexyl.

The factor of *ca.* 35 between the rates of acetolysis of the *cis*- and *trans*-toluenesulfonates suggests that ionization of the *cis*-toluenesulfonate III is somewhat anchimerically accelerated. The occurrence of the special salt effect in acetolysis of the *cis*-toluenesulfonate is diagnostic[6b] for the occurrence of an ion pair with a special structure for the cation, which evidently reacts stereospecifically with solvent.

That the cation from toluenesulfonate III does indeed have the non-classical structure II is suggested by the behavior of 3-deuterated bicyclohexyl toluenesulfonate V-D, m.p. 51.5–52.0°, prepared from deuterated alcohol from litlhium aluminum deuteride reduction of the bicyclohexanone. The infrared spectrum of the alcohol from acetolysis of V-D shows that the product alcohol now has deuterium on the cyclopropane ring, the corresponding C-D stretching vibration occurring at 2266 cm.$^{-1}$. Analogously, the intensity of the C-D stretching absorption at 2155 cm.$^{-1}$, the major absorption band for the deuterium on the carbinol carbon atom, is much decreased, the absorption in the product alcohol being 33 ± 1% as large as that in the parent deuterated alcohol. Although the analysis is less accurate, proton magnetic resonance spectra are consistent with the 2:1 ratio of cyclopropane ring deuterium to carbinol carbon deuterium in the product. It is quite clear that the deuterium scrambling during acetolysis of the labelled toluenesulfonate V-D corresponds exactly to expectations based on the non-classical cation II.

Because of an analogy with the cyclopropenyl cation, II may be named the "tris-homocyclopropenyl" cation.

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(2) Satisfactory carbon and hydrogen analyses were obtained for all the new compounds here mentioned.

(3) H. E. Simmons and R. D. Smith, THIS JOURNAL, **81**, 4256 (1959).

(4) S. Winstein, E. L. Allred and J. Sonnenberg, *ibid.*, **81**, 5833 (1959).

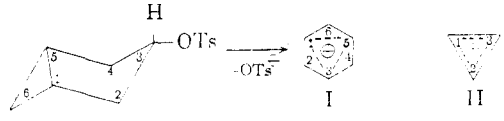
(5) K. Hofmann, *et al.*, *ibid.*, **81**, 992 (1959).

(6) (a) S. Winstein, *et al.*, THIS JOURNAL, **76**, 2597 (1954); *Chemistry and Industry*, 664 (1954); (b) S. Winstein, *Experientia Supplementum*, II, 137 (1955).

HOMO-AROMATIC STRUCTURES

Sir:

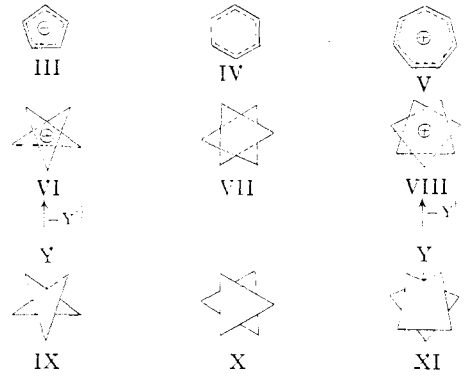
There is evidence[1] that the 3-bicyclo[3.1.0]hexyl cation has the nonclassical structure I. Except that orbital overlap is not π and the 1,3 overlap and



I II

exchange integrals are smaller in magnitude in cation I than the 1,2 integrals in the cyclopropenyl cation II, the situation in the non-classical cation I is wave-mechanically[2] quite analogous to that in the cyclopropenyl cation II. For this reason the term "tris-homocyclopropenyl" is suggested[1] for cation I.

Cation I is the non-classical tris-homo counterpart of the cyclopropenyl cation II, one of the species which fits Hückel's $(4n + 2)\pi$-electron rule for conjugated monocyclic systems.[2] Conceptually, the homo relationship of non-classical ion I to the cyclopropenyl cation II may be generalized to include homo counterparts of other examples of the $(4n + 2)$ rule, such as cyclopentadienide ion III, benzene IV, and tropylium ion V. They are pentahomocyclopentadienide ion VI, $C_{10}H_{15}^{\ominus}$, hexahomobenzene VII, $C_{12}H_{18}$, and heptahomotropylium ion VIII, $C_{14}H_{21}^{\ominus}$. Species VI could be visualized to arise from a material such as IX, structure X would represent one of the "Kekule" structures for hexahomobenzene, and the heptahomotropylium ion VIII could be formulated as the product of ionization of a material with structure XI.



(1) S. Winstein, J. Sonnenberg and L. deVries, THIS JOURNAL, **81**, 6523 (1959).

(2) (a) E. Hückel, *Z. Physik*, **70**, 204 (1931); (b) J. D. Roberts, A. Streitwieser, Jr., and C. M. Regan, THIS JOURNAL, **74**, 4579 (1952).

It is not easy to anticipate whether the balance between quantum-mechanical delocalization energy and the compression energy necessary to force classical structures (like the "Kekule" structures of type X) into the same geometry will indeed place species VI–VIII in the category of homo-aromatic mesomeric compounds. While quantum-mechanical delocalization energies in cases VI–VIII will be much less than in cases III–V, we probably can expect compression energies to be less also. The balance between compression energy and quantum-mechanical delocalization energy in cases VI–VIII appears to be sufficiently analogous to the one for I, that the observed results with I warrant experiments to test for the occurrence of homo-aromatic structures VI–VIII. These are being undertaken.

Examples of homoconjugation have been discussed previously, such as the homoallylic[3a] cation, the *anti*-7-norbornenyl cation[3b] (termed a bishomocyclopropenyl cation by Roberts[3c]) and the "planar pseudo-aromatic structure" for tropilidene visualized by Doering.[4] The latter example could be called monohomobenzene. Cases VI–VIII discussed above differ from these by having complete equivalence of the classical contributing structures. Perhaps "perhomo-aromatic" is a useful term for VI–VIII.

(3) (a) *E.g.*, M. Simonetta and S. Winstein, THIS JOURNAL, **76**, 18 (1954); (b) S. Winstein, M. Shatavsky, C. Norton and R. B. Woodward, *ibid.*, **77**, 4183 (1955); (c) W. G. Woods, R. A. Carboni and J. D. Roberts, *ibid.*, **78**, 5653 (1956).

(4) W. E. Doering, *et al.*, *ibid.*, **78**, 5448 (1956).

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REQUIREMENT FOR FLAVIN COENZYME IN THE ENZYMATIC SYNTHESIS OF METHIONINE IN VITRO[1]

Sir:

Several investigators have studied the biosynthesis of the methyl group of methionine in cell-free systems with serine or formaldehyde as donors and homocysteine as acceptor of the one-carbon fragment.[2-6] In extracts of certain mutants of *Escherichia coli*, cofactor requirements were shown previously for pyridoxal phosphate, tetrahydrofolic acid, adenosine triphosphate, DPNH[7] and vitamin B_{12}.[2,3,8] Recently in this laboratory a requirement for FAD or FMN has been demonstrated.

The effect of the flavin coenzymes has been shown with a system consisting of these three purified enzyme fractions obtained from *E. coli* mutant 113-3,[9] which requires vitamin B_{12} or methionine for growth: (1) serine hydroxymethylase, (2) a vitamin B_{12}-containing enzyme[3,8] and (3) a fraction partially purified by ammonium sulfate precipitation and chromatography on adsorbents. The vitamin B_{12}-containing enzyme was purified about 60-fold by means of ammonium sulfate fractionation and then by adsorption and elution from calcium phosphate gel and by chromatography on diethylaminoethyl cellulose and hydroxylapatite.

The enzymatic system carried out methionine synthesis in the presence of the protein fractions and all of the indicated cofactors (Table I). The addition of reduced flavin compounds to incubation mixtures obviated the requirement for DPNH when incubation was carried out under hydrogen.

TABLE I

All vessels contained per ml.: potassium phosphate buffer, *p*H 7.2, 50–100 μmoles; L-serine, 5–10 μmoles; L-homocysteine, 10 μmoles; pyridoxal phosphate, 0.25 μmole; adenosine triphosphate, 5 μmoles; Mg^{++}, 10 μmoles; tetrahydrofolic acid, 0.5 μmole; serine hydroxymethylase; B_{12}-containing enzyme; and third enzyme fraction. To this basic system these additions were made when indicated: DPNH, 2 μmoles; oxidized FAD, 0.16 μmole; reduced FAD or FMN (catalytic hydrogenation with 30% palladium on charcoal), 0.2 μmole. Incubation was for 2 or 3 hours at 37°. Methionine was assayed microbiologically with *Leuconostoc mesenteroides* P 60.

Expt.	Additions	Gas phase	Methionine synthesized mμmoles
A	DPNH, oxidized FAD	N_2	71
	DPNH	N_2	11
	None	N_2	7
B	Reduced FMN	H_2	592
	Reduced FAD	H_2	388
	Filtered catalyst suspension (without flavin compounds)	H_2	42
C	Reduced FMN	H_2	236
	Reduced FMN	He	34

It is believed that the high values for methionine synthesis obtained were due to regeneration of reduced flavin by hydrogen gas catalyzed by traces of palladium which escaped filtration. Incubation under helium resulted in very little methionine formation. These preliminary results suggest that the requirement for pyridine dinucleotide in methionine biosynthesis can be explained by its role in reducing the flavin component of the system.

(10) American Heart Association Advanced Research Fellow.

(11) Karl Taylor Compton Fellow of the Nutrition Foundation in Biochemistry.

(12) United States Public Health Service Predoctoral Fellow.

(13) National Science Foundation Predoctoral Fellow.

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IDENTIFICATION OF A STEROL WITH ACRASIN ACTIVITY IN A SLIME MOLD

Sir:

Acrasin is a chemotactic hormone produced by individual amoeboid cells of the slime mold, *Dictyostelium discoideum*.[1,2,3] In response to a

(1) This work was supported in part by grants from the National Science Foundation and the Greater Boston Chapter of the Massachusetts Heart Association.

(2) C. W. Helleiner and D. D. Woods, *Biochem. J.*, **63**, 26P (1956).

(3) F. T. Hatch, S. Takeyama and J. M. Buchanan, *Federation Proc.*, **18**, 243 (1959).

(4) V. M. Doctor, T. L. Patton and J. Awapara, *Arch. Biochem. Biophys.*, **67**, 404 (1957).

(5) A. Nakao and D. M. Greenberg, *J. Biol. Chem.*, **230**, 603 (1958).

(6) A. Stevens and W. Sakami, *ibid.*, **234**, 2063 (1959).

(7) Abbreviations used are: DPNH, reduced diphosphopyridine nucleotide; FAD, flavin adenine dinucleotide; FMN, riboflavin-5-phosphate.

(8) R. L. Kisliuk and D. D. Woods, *Federation Proc.*, **18**, 261 (1959).

(9) B. D. Davis and E. S. Mingioli, *J. Bacteriol.*, **60**, 17 (1950).

(1) E. H. Runyan, *Collecting Net. Woods Hole*, **17**, 88 (1942).

(2) J. T. Bonner, *J. Exptl. Zool.*, **106**, 1 (1947).

(3) B. M. Shaffer, *Nature*, **171**, 957 (1953).