water by volume. Ultraviolet absorptions support the bonding of the nitrogen system to nickel in the complex as maxima are observed at 252, 268, 345 and 585 mμ with respective molar extinction coefficients of 14,000, 13,050, 7550 and 6200, in 95% ethanol. This contrasts with the spectrum of azobenzene which has maxima at 230, 318 and 440 mμ with molar extinctions of 8370, 19,500 and 587. The loss of absorption in the 318-mμ region, attributed to the conjugation of the unsaturated nitrogen with the phenyl rings, strongly suggests that the nitrogen system in the complex is bonded to nickel in a manner shown in structure I and this bonding allows nickel to attain rare gas structure.

The accumulated experimental evidence is in agreement with the proposed structure of the isolated nickel complex. Although the position of the deuterium label in the liberated azobenzene following deuteride reduction was not determined, it is difficult to visualize, on the basis of steric requirements, bonding between nickel and the phenyl ring at any site other than the *ortho* position. The exact position of nickel relative to the nitrogen system also has not been established, as such refinements can only be determined unequivocally with X-ray diffraction studies.

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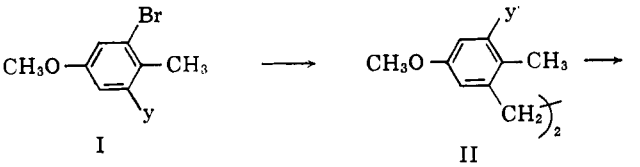
2,7-DIACETOXY-*trans*-15,16-DIMETHYL-15,16-DIHYDROPYRENE. A NOVEL AROMATIC SYSTEM WITH METHYL GROUPS INTERNAL TO THE π-ELECTRON CLOUD[1]

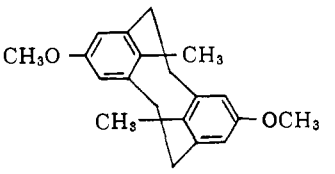
Sir:

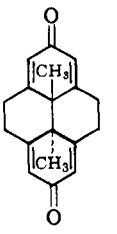
We wish to report the results of an investigation bearing on two classical problems in the chemistry of aromatic molecules: (1) the question of aromaticity of monocyclic polyenes larger than benzene; and (2) the possibility of having functional groups within the cavity of an aromatic π-electron cloud. The general class of compounds which was chosen for study was that of the *trans*-15,16-dihydropyrenes,[2,3] and the present communication describes the synthesis of 2,7-diacetoxy-*trans*-15,16-dimethyl-15,16-dihydropyrene (VI), a molecule fulfilling both of the requirements previously mentioned.

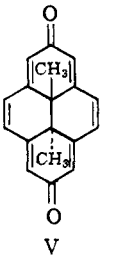
The synthesis of VI required seventeen steps and utilized the metacyclophane III as a key intermediate to provide the correct geometry for the final stages. The path taken is presented by listing the intermediate compounds in schematic outline. Each of the steps down to II (y = $-CH_2I$) proceeded in high yield following the general methods outlined previously.[2] The cyclization of II (y = $-CH_2I$) to give III, m.p. 212.5–213.0, occurred in 55% yield using sodium and tetraphenylethylene and the structure of III is supported by molecular weight data and by the close correspondence of its n.m.r. spectrum to that of 4,12-dimethyl-(2,2)-metacyclophane.[4,5] Oxidation of III with ferric chloride in chloroform gave directly in 92% yield the bis-dienone IV, m.p. 260° dec., which on treatment with N-bromosuccinimide produced the yellow quinone V, m.p. 265° dec., in 76% yield. That V was a true quinone was indicated by the appearance of a deep blue-green color when it was treated with dithionite. Further, a preparative reduction of V with zinc and acetic anhydride in the presence of triethylamine effected the separation of brilliant green crystals of the hydroquinone diacetate VI, m.p. 204.5–205.0° softening at 199°, λ_{max} (ε) (cyclohexane) 643 (1,840), 466 (10,250), 371 (38,900) and 337 mμ (97,400), in high yield. As would be expected, hydrolysis of VI and oxidation regenerated the quinone V.

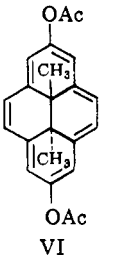


y	M.p., °C.	y	M.p., °C.
-Br[6]	47–48	-Br	143.5–144.0
-CN	91–93	-CN	195–196
$-CO_2H$	160–161	$-CH_2NH_2$	94.5–95.0
$-CO_2CH_3$	42.5–43.5	$-CH_2OH$	118–118.5
$-CH_2OH$	69.0–69.5	$-CH_2Br$	185.5–186.0
$-CH_2Br$	72.0–72.5	$-CH_2I$	147 dec.









Probably, the most pertinent evidence regarding VI is its n.m.r. spectrum which shows two equal sharp signals at 1.42 and 1.63 τ (8 ring protons), a singlet at 7.50 τ (6 protons of the acetate methyls) and a singlet at 14.03 τ (6 protons of the internal methyls). Thus, the very strong ring current which is clearly evident substantiates the conclusion that VI is aromatic. This result is particularly interesting in view of the elegant work of Sondheimer and his collaborators on the closely related annulenes.[7,8]

The presence or absence of a ring current has been the basis for describing (18)annulene and 1,8-bisdehydro(14)annulene as aromatic but (14)annulene as a non-aromatic polyene. The good agreement between the ultraviolet and visible spectra of VI and 1,8-bisdehydro(14)annulene is in keeping with the presence of a 14-π electron aromatic cloud in each and is further indication that the lack of aromaticity in (14)annulene is due to distortion by the internal protons.

Actually, VI is quite stable toward heat, light and air, suggesting that it is also "aromatic" in the classical, non-thermodynamic sense. Again, this is in agreement with the properties of 1,8-bisdehydro(14)annulene but contrasts with (18)annulene in which presumably there is still appreciable repulsion among the internal hydrogens. It should be pointed out that since the

(1) The authors gratefully acknowledge financial support by the National Science Foundation.

(2) W. S. Lindsay, P. Stokes, L. G. Humber and V. Boekelheide, *J. Am. Chem. Soc.*, **83**, 943 (1961).

(3) R. W. Griffin, Ph.D. Thesis, University of Rochester, 1960.

(4) D. J. Wilson, V. Boekelheide and R. W. Griffin, *J. Am. Chem. Soc.*, **82**, 6302 (1960).

(5) A. W. Hanson, *Acta Cryst.*, **15**, 956 (1962).

(6) The starting material (I, y = −Br) was prepared by methylation of the corresponding phenol (G. Baddeley and J. Plant, *J. Chem. Soc.*, 525 (1943)).

(7) F. Sondheimer, Y. Gaoni, L. M. Jackman, N. A. Bailey and R. Mason, *J. Am. Chem. Soc.*, **84**, 4595 (1962).

(8) F. Sondheimer, R. Wolovsky and Y. Amiel, *ibid.*, **84**, 274 (1962).

spectral evidence supports structure VI rather than its metacyclophane valence tautomer, the delocalization energy of VI probably is in excess of that of two isolated benzene rings.

The synthesis of VI raises the possibility that a variety of such molecules can be prepared to test experimentally the exact nature of an aromatic π-electron cloud with regard to various physical and chemical properties such as steric hindrance, unusual bonding, and unusual interactions with ions or radicals generated within the π-electron cavity. Exploration of these general questions is being undertaken.[9]

(9) The analytical and spectral data of all of the compounds described are in accord with the assigned structures.

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STUDIES ON POLYPEPTIDES. XXVII. ELIMINATION OF THE METHIONINE RESIDUE AS AN ESSENTIAL FUNCTIONAL UNIT FOR *in vivo* ADRENOCORTICOTROPIC ACTIVITY[1-3]

Sir:

Exposure to hydrogen peroxide lowers markedly the adrenal-stimulating activity of pig corticotropin and incubation with thiols brings about essentially complete reactivation of oxidized material.[4-8] This phenomenon is also observed with the skin darkening hormones α- and β-MSH and with parathyroid peptides.[9-11] All these hormones contain methionine as the sole sulfur containing residue, and reversible oxidation of the thioether sulfur to the sulfoxide appears to provide the basis for the deactivation–reactivation behavior.[10,12]

The non-essential nature of the methionine residue as concerns melanocyte expanding activity follows from the observation that a family of peptides related to the N-terminus of the α-MSH sequence possesses the ability to darken frog skin although they do not contain methionine.[13]

In order to clarify the essential nature of the methionine residue for adrenocorticotropic activity, we synthesized the eicosapeptide amide seryltyrosylseryl-α-amino-*n*-butyrylglutamylhistidylphenylalanylarginyltryptophylglycyllysylprolylvalylglycyllysyllysylarginylarginylprolylvaline amide (I) and evaluated its *in vivo* adrenocorticotropic activity in the rat. Peptide I is the α-amino-*n*-butyric acid analog of the adrenocorticotropically fully active eicosapeptide amide which corresponds to the N-terminal half of the pig corticotropin molecule.[14] Evaluation of the adrenocorticotropic activity of three different batches of I by the rat adrenal ascorbic acid depletion method[15] gave values of 31.2 ± 4.2, 37.8 ± 6.6, 39.2 ± 7.2 and 48.4 ± 10.4 IU/mg. Of considerable practical importance is the finding that peptide I appears to exhibit 0.7 times the i.v. potency when administration is by the subcutaneous route; values of 32.4 ± 3.0, 29.4 ± 3.0, 24.3 ± 3 and 21.6 ± 3.3 were obtained in four assays. Preliminary studies have shown peptide (I) to exhibit 30–40% the *in vitro* and approximately 35% the *in vivo* steroidogenic activity of corticotropin A_1 on a weight basis.[16] The melanophoretic activity of I is 1.6×10^7 MSH units/g.[17]

These findings exclude the methionine residue as a "functionally active site"[18] for adrenocorticotropic activity and suggest that oxidation of the methionine sulfur lowers markedly the binding affinity of corticotropin and its biologically active fragments for the receptor with a corresponding decrease in biological activity. The fact that the sulfoxide or sulfone of a biologically active peptide containing methionine may possess a significantly lower physiological activity than the genuine material appears to provide little information regarding the "functional" importance of the methionine residue.

For the synthesis of I, N-*t*-butyloxycarbonylseryltyrosylseryl-α-amino-*n*-butyrylglutamine (II) (dihydrate; *Anal.* Found: C, 50.0; H, 6.9; N, 12.3; m.p. 146–148°; $[\alpha]^{27}D$ −28.6° in methanol; R_f^1 0.73[19]) was coupled with histidylphenylalanylarginyltryptophylglycine[20] *via* the azide to give N-*t*-butyloxycarbonylseryltyrosylseryl-α-amino-*n*-butyrylglutaminylhistidylphenylalanylarginyltryptophylglycine (III) (monoacetate trihydrate; *Anal.* Found: C, 53.4; H, 6.7; N, 15.9; $[\alpha]^{27}D$ −27.2° in 50% v./v. acetic acid; R_f^1 0.53; amino acid ratios in acid hydrolysate $ser_{2.09}tyr_{1.00}but_{1.09}glu_{0.94}his_{1.00}phe_{0.96}arg_{1.00}gly_{0.96}$). The acetate (III) was converted to the hydrochloride which was then treated with the hydrochloride of N^{ϵ}-*t*-butyloxycarbonyllysylprolylvalylglycyl-N^{ϵ}-*t*-butyloxycarbonyllysyl-N^{ϵ}-*t*-butyloxycarbonyllysylarginylarginylprolylvaline amide (triacetate hexahydrate; *Anal.* Found: C, 50.0; H, 8.2; N, 16.3; $[\alpha]^{27}D$ −71.8° in 10% v./v. acetic acid; R_f^1 0.68; amino acid ratios in acid hydrolysate $lys_{3.06}pro_{1.94}val_{2.02}gly_{1.00}arg_{1.98}$) using N,N′-carbonyldiimidazole[21] as the condensing reagent. The ensuing protected eicosapeptide amide *t*-butyloxycarbonylseryltyrosylseryl-α-amino-*n*-butyrylglutaminylhistidylphenylalanylarginyltryptophylglycyl-N^{ϵ}-*t*-butyloxycarbonyllysylprolylvalylglycyl-N^{ϵ}-*t*-butyloxycarbonyllysyl-N^{ϵ}-*t*-butyloxycarbonyllysylarginylarginylprolylvaline amide (IV) (triacetate hydrate; $[\alpha]^{26}D$ −54.7° in 10% v./v. acetic acid; single ninhydrin nega-

(1) The authors wish to express their appreciation to the U. S. Public Health Service and the National Science Foundation for generous support of this investigation.

(2) The peptides and peptide derivatives mentioned are of the L-configuration. In the interest of space conservation the customary L-designation for individual amino residues is omitted.

(3) See *J. Am. Chem. Soc.*, **85**, 833 (1963), for paper XXVI in this series.

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(5) H. B. F. Dixon, *Biochim. Biophys. Acta*, **18**, 599 (1955).

(6) H. B. F. Dixon and M. P. Stack-Dunne, *Biochem. J.*, **61**, 483 (1955).

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(10) K. Hofmann and H. Yajima, in "Recent Progress in Hormone Research," G. Pincus, Ed., Vol. 18, Academic Press Inc., New York, N. Y., 1962, p. 41.

(11) H. Rasmussen and L. C. Craig, *ibid.*, p. 269.

(12) M. L. Dedman, T. H. Farmer and C. J. O. R. Morris, *Biochem. J.*, **78**, 348 (1961).

(13) See K. Hofmann, *Ann. Rev. Biochem.*, **31**, 213 (1962), for tabulation of a series of melanophoretically active peptides.

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(15) Ascorbic acid depleting activity was determined in 24-hr. hypophysectomized rats according to the method of "U. S. Pharmacopeia," Vol. XV, against the U.S.P. reference standard. We are indebted to Dr. Joseph D. Fisher of Armour Pharmaceutical Company, Kankakee, Illinois, for these biological determinations.

(16) We wish to express our gratitude to Drs. F. L. Engel and H. E. Lebovitz of Duke University Medical School for these assays.

(17) We wish to express our thanks to Dr. A. B. Lerner of Yale University School of Medicine, for the MSH assays which were performed according to the method of K. Shizume, A. B. Lerner and T. B. Fitzpatrick, *Endocrinol.*, **54**, 553 (1954).

(18) See ref. 13 for definition of this term.

(19) R_f^1 values refer to the Partridge system (S. M. Partridge, *Biochem. J.*, **42**, 238 (1948); R_f^2 values refer to the system 1-butanol, pyridine, acetic acid, water 30:20:6:24 (S. G. Waley and J. Watson, *ibid.*, **55**, 328 (1953)). With the latter system R_f values are expressed as multiples of the distance traveled by a histidine marker.

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(21) (a) H. A. Staab, *Ann. Chem.*, **609**, 75 (1957); (b) G. W. Anderson and R. Paul, *J. Am. Chem. Soc.*, **80**, 4423 (1958); (c) R. Paul and G. W. Anderson, *ibid.*, **82**, 4596 (1960).