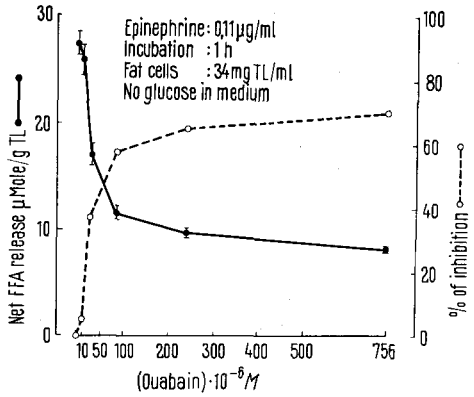
ACTH or glucagon, as illustrated in the Table. This Table also shows that, even in the absence of ouabain, the lipolytic activity of ACTH, epinephrine, or glucagon was markedly decreased by the omission of potassium from the incubation medium. These observations suggest that the inhibitory effect of ouabain on FFA release is a general phenomenon and, furthermore, that it is mimicked by removal of potassium from the medium. It would seem likely, therefore, that the mechanism of FFA release from isolated fat cells is linked to the Na^+ and K^+ related steps of active transport.



Dose response curve of ouabain on inhibition of net FFA change stimulated by epinephrine.

Comparison of the inhibitory effect of ouabain and of K^+-free medium on FFA mobilization from isolated fat cells. Mean of 4 ± S.E., expressed as µmoles/g TL/h. No glucose in medium

Additions	K^+ in buffer	ACTH (2 mU/ml)	Epinephrine (0.1 µgm/ml)	Glucagon (1 µgm/ml)
Control	+	9.7 (4.0)	3.75	0.46 (0.15)
Hormones	+	55.3 (6.2)	37.50 (9.1)	18.40 (1.3)
Hormones + ouabain	+	13.2 (5.2)[a]	6.82 (4.3)[b]	11.80 (2.3)[b]
Control	−	5.2 (2.7)	1.16	−
Hormones	−	10.1 (2.4)	3.16 (2.5)	11.10 (1.4)

[a] Ouabain $5.5 \cdot 10^{-4} M$. [b] Ouabain $2.4 \cdot 10^{-4} M$.

It remains to be seen whether the site of the inhibitory effect of ouabain on FFA release is the chain of reactions leading to the activation of a hormone-sensitive lipase or a still hypothetical active FFA transport through the cell membrane, or both. As we have shown in very recent experiments that ouabain also significantly inhibits the FFA release induced in fat cells by N^6C'-2-dibutyril-cyclic-3′,5′-AMP[7] as well as epinephrine-stimulated glycogenolysis, the most likely present hypothesis is that of an inhibitory ouabain effect on both adenyl cyclase and on membrane ATP-ase. The former enzyme is related to the activation of the hormone-sensitive lipase[8] while the latter[9] is well known to be linked to active transport systems in membranes[10].

Résumé. La mobilisation des acides gras libres (FFA) induite par les hormones lipolytiques dans les cellules adipeuses isolées est inhibée par l'adjonction d'ouabaïne. Le transport des FFA hors de la cellule semble lié en partie à la pompe Na^+-K^+. Une hypothèse sur le mode d'action de l'ouabaïne est discutée.

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[7] N^6C'-2-dibutyryl-cyclic-3′-5′-AMP was obtained through the courtesy of Professor Th. Posternak, Ecole de Chimie, Université de Genève, (Switzerland).

[8] R. W. Butcher, R. J. Ho, H. C. Meng, and E. W. Sutherland, abstracts of 6th International Congress of Biochemistry (New York 1964), p. 173.

[9] J. C. Skou, Physiol. Rev. *45*, 596 (1965).

[10] The authors are indebted to Miss A. Eisold and Miss E. Keller for their skilful technical help.

The Occurrence of 4-Ethyl- and 2,5-Dimethylazulene in Cracking Column Products

Some time ago we received small samples of intensely blue coloured distillates (Table I), products from a cracking column at Grangemouth(Scotland), from which we isolated three azulenes (see Table I). Dilution of the distillate fractions with petroleum ether (b.p. 40–60°), extraction with ice-cold phosphoric acid, and regeneration of the acid-soluble material gave blue oils comprising about 0.5% of the original distillate fraction. Chromatography on alumina (petroleum ether-benzene 9:1) showed that each was homogeneous. The compounds, indicated by I, II and III, were characterized by their visible spectra[1] in hexane (Table II) and by their crystalline trinitrobenzene (TNB) complexes.

Elemental analysis of the TNB-complex of III, brown needles (from ethanol) m.p. 141–142°, showed that III was a $C_{12}H_{12}$ hydrocarbon, i.e. an ethyl- or dimethylazulene; found, 58.4% C; 3.9% H; 11.8% N ($C_{18}H_{15}N_3O_6$ requires 58.5% C; 4.1% H; 11.4% N).

The fine structure in the visible spectrum of compound III (Table II) had an average hypsochromic displacement of 17 nm with respect to the corresponding maxima for azulene. This indicated, according to the Plattner rules on the effect of alkyl substitution on the visible spectra of azulenes[2], as only possible structures for III 2-ethyl-[3],

[1] Measured on a Unicam SP 500 spectrophotometer.

[2] See E. Heilbronner, Tetrahedron *19*, supplement 2, 289 (1963), and refs. 18 and 19 contained therein.

[3] Pl. A. Plattner and A. Fürst, Helv. Chim. Acta *28*, 1636 (1945).

4-ethyl-[4], or 6-ethylazulene[5]. The characteristic visible spectrum together with the elemental analysis and m.p. of the TNB complex of III identify it unequivocally as 4-ethylazulene (TNB complex, lit. m.p. 146–147°)[4].

TREIBS has shown that 4,7-dimethylazulene is a side-product in the catalytic dehydrogenation of butane to butene[6]. He has discussed the mode of formation of this $C_{12}H_{12}$ hydrocarbon from three C_4 hydrocarbon units, and the similar formation of a $C_{15}H_{18}$[7] (trialkyl) azulene from three C_5 hydrocarbon (pentanes and pentenes) units[8], discounting the possibility of their formation from smaller hydrocarbon units in the process. Compound II formed a TNB complex, brown needles (from ethanol) m.p. 149 to 151° (found, 11.9% N)[10]. The visible spectrum of II had an average hypsochromic displacement of only 3 nm relative to that of azulene. This suggested a dimethylazulene with the methyl groups in positions such that their effects on the visible spectrum approximately cancelled, i.e. 2,5-[10], 4,5-[11], 4,7-[6] or 5,6-dimethylazulene. The visible spectral data and the m.p. of the TNB complex of II show clearly that it is 2,5-dimethylazulene (TNB complex, lit. m.p. 149-150.5°)[10].

The structure of compound I remains uncertain. We assume from TREIBS' work and from its occurrence with II and III, that I is isomeric with these. The high proportion of C_4 hydrocarbons formed in the catalytic cracking of *n*-hexadecane[12] may support this view. The visible spectrum of I like that of II indicates, therefore, a dimethylazulene with the methyl groups again in positions where their effects are neutralized, i.e. 4,5-, 4,7- or 5,6-dimethylazulene, since I is not identical with II. Compound I formed a TNB complex[13], brown needles (from ethanol), m.p. 138–139°. Therefore it cannot be 4,5-dimethylazulene (TNB complex, m.p. 157.5°)[11] nor 4,7-dimethylazulene (TNB complex, m.p. 161–162°)[6]. Comparison of the spectral data confirms this. We suggest that Compound I may be 5,6-dimethylazulene, which has not yet been described in the literature, possibly because of the relative inaccessibility of 5,6-dimethylindan[14] for the usual synthesis with diazoacetic ester. However, with the exception of the two maxima at longer wave-length, the visible spectrum of compound I is in fair agreement with that of the analogous 5,6-tetramethyleneazulene[16].

The materials which were not extracted by phosphoric acid (above) were heated with sulphur at 200°. None of them yielded further amounts of azulenes, in agreement with similar observations of TREIBS[6]. They contain, therefore, no azulene precursors since these, particularly in a diluent, are dehydrogenated in good yield by this treatment[17].

The novel features of the work described here, we consider, are the diversity of azulenes formed during the cracking process, and the fact that each of the distillate fractions examined contained only one azulene[18,19].

Zusammenfassung. Es wird das Vorkommen des 4-Äthyl- und des 2,5-Dimethylazulens in hochsiedenden Nebenprodukten des thermischen Crack-Verfahrens festgestellt. Die Azulene wurden durch ihre Spektren im Sichtbaren, sowie durch ihre Trinitrobenzolkomplexe charakterisiert.

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Table I. Cracking column products

Fraction (°C) at 50 mm	n_D^{20}	Compound isolated
236–238	1.6032	I
238–243	1.5970	II
266–273[a]	1.5976	III

[a] Other fractions in the range 243–266° were coloured blue, but there was insufficient material at our disposal for characterization.

Table II. Visible spectra (λ_{max} in nm)

Azulene	I	II	2,5-Dimethylazulene[10]	III	4-Ethylazulene[4]
700	685	692	693 668	670	674
660	640	655	655	640	640
630	625	626	628	612	615
600	595	601	603	586	587
580	572	574	578	565	566
555	552	556	558	545	545
536	532	535	545	525	

[4] K. HAFNER and H. WELDES, Annalen *606*, 90 (1957).
[5] K. HAFNER, H. PELSTER, and H. PATZELT, Annalen *650*, 80 (1961).
[6] W. TREIBS and R. KLIMKE, Annalen *586*, 212 (1954).
[7] Spectral and other evidence favoured 1,4-dimethyl-8-isopropylazulene for the structure of this compound[8]. The TNB complex has the same m.p. as that of 1,4-dimethyl-8-isopropylazulene TNB complex[9].
[8] R. KLIMKE and W. TREIBS, Annalen *598*, 46 (1956).
[9] N analysis of TNB complex of III. The TNB complex of a (mono) methylazulene, $C_{17}H_{13}N_3O_6$, requires 11.8% N. The visible spectrum of II, however, is incompatible with that of the (mono) methylazulenes[2]. There was insufficient material for complete elemental analysis.
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[17] D. H. REID, W. H. STAFFORD, and J. P. WARD, J. Chem. Soc. 1100 (1958).
[18] Acknowledgment: We thank British Hydrocarbon Chemicals Ltd. for samples, and Mr. J. HABESHAW for information on their source.
[19] Relevant to the mechanism of formation is a review (H. PINES and C. T. GOETSCHEL, J. Org. Chem. *30*, 3530, 1965) in which the participation of 7-membered ring species in the aromatization of alkanes over chromia-alumina catalysts is discussed.
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