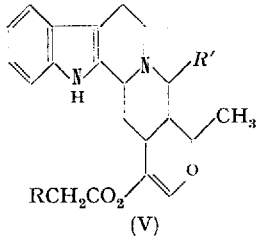
We find (C)Me 5·78, 6·37%; active H, 0·62% [2(C)Me requires 7·7 and 2 H requires 0·52%].

The I.R. absorption spectrum includes bands at 2·82, 3·00 and 3·28 μ (OH and NH), 5·86 μ and 5·94 μ (ester or ketone carbonyl group conjugated with a double bond), 6·14 μ (asymmetrically substituted double bond) and 13·50 μ (o-disubstituted benzene nucleus). This evidence, together with the U.V. is consistent with the presence of $MeO_2C—C=C—O—$, although the extra conjugated carbonyl peak in the I.R. is an unusual feature. There is an "extra" carbon atom, probably corresponding to a (C)Me, and the constitution (V) is feasible (R = H, R' = Me, or R = Me, R' = H), this structure also showing the suggested[1] relation to mayumbine.



(V)

Akuammiline ($C_{22}H_{24}O_4N_2$) contains 1 OMe and no NMe[2]. It appears to contain two (C)Me groups (7·0%, theory, 7·9%), and its U.V. spectrum is in the main like that of an indole, although it presents some slight variations. The first maximum at 2,200 A (logε, 4·25) is about 50 A shorter, and the second peak ($\lambda_{max.}$ 2,400 A; logε, 3·54) is rather broader than usual. The I.R. absorption shows a broad and very weak band at 2·9 μ (OH or NH or not significant), bands at 5·76 μ (C = O of unconjugated ester type), at 6·16 μ (probably C = C but could be C = N), at 6·26 μ (more conjugated C = C), at 13·29 μ (o-disubstituted benzene, but this is uncertain because bands at 12·90 and 13·21 μ are also observed).

Akuammiline could be a keto-akuammigine or a hydroxydehydroakuammigine. The modified indole spectrum suggests that a functional group is attached to the indole nucleus. The base gives a bright crimson red Otto reaction which is characteristic.

Akuammicine ($C_{19}H_{22}O_2N_2$) is said to contain 1 OMe and no NMe (but *v.infra*). In many ways it is *sui generis* with the remarkably high rotatory power $[\alpha]_D$ – 737·5° (in alcohol)[2]. Its Otto reaction is an intense and persistent royal blue colour.

The U.V. spectrum differs fundamentally from the spectra of the congeneric alkaloids. It is almost identical with that given by echitamidine[3] (which also has a high rotatory power[4]) and suggests considerable conjugation.

Among strychnine derivatives, benzylidenestrychnine exhibits a deep blue Otto reaction, and high rotatory powers are observed with a number of substances in the group which have double bonds in conjugation with the amide carbonyl. We may be sure that akuammicine contains the part structure —N—CO—C=C, with an asymmetric centre vicinal to an unsaturated group.

(C)Me determination gave Me 6·15, 6·6% [2(C)Me requires 9·68%].

The I.R. absorption spectrum has bands at 2·99 μ (OH or more probably NH), 6·03 μ (typical amide carbonyl, probably with further conjugation), 6·24 μ (probably armoatic double bond), 13·39 μ (o-disubstituted benzene, supported by absence of bands at 12·3 and 12·8 μ).

In the case of echitamidine[1] about half of the methyl was obtained as OMe (Zeisel) and half as NMe (Herzig-Meyer), but there is only one methyl group, and hence the (O)Me is unusually hard to remove, or the (N)Me is unusually labile. We prefer the latter hypothesis, which was noted by Goodson as a possibility, because COOMe cannot be present in akuammicine and it is hard to accomodate a methoxyl group otherwise.

M. F. Millson, R. Robinson and A. F. Thomas

The Dyson Perrins Laboratory University of Oxford, December 17, 1952.

Zusammenfassung

Für das Alkaloid *Akuammin* wird die neue Bruttoformel $C_{22}H_{26}O_4N_2$ aufgestellt und durch Vergleich verschiedener UV.- und IR.-Spektren und Reaktionen das Vorliegen einer 5-Hydroxydihydroindol-Verbindung abgeleitet. Die Zinkstaubdestillation liefert 3-Äthyl-Pyridin und vermutlich Skatol. Als Arbeitshypothese wird für *Akuammin* die Formel (III) vorgeschlagen. Die UV.- und IR.-Spektren und Reaktionen von Pseudo-akuammigin, Akuammigin, Akuammilin und Akuammicin sind gleichfalls diskutiert.

[1] J. A. Goodson, J. Chem. Soc. *1932*, 2626.

The Origin of Steric Hindrance in Cyclohexane Derivatives

Hassel[1] and Pitzer[2] and coworkers have pointed out that in the stable or "chair" form of cyclohexane there are two types of bonds, so-called equatorial ($\varkappa$ or e) and polar (ε or p) bonds. On the basis of this concept, Barton[3] has been able to make important and far-reaching predictions on the reactivity of substituted cyclohexanes and natural products containing cyclohexane rings. One of these predictions is concerned with the relative ease of esterification of hydroxyl groups and ease of hydrolysis of ester groups in polar and equatorial positions. Hydroxyl groups are esterified more easily and ester groups hydrolyzed more easily when in the *equatorial* than when in the *polar* position, due to the greater steric hindrance in the latter. The reason for this might be twofold. It is possible that an ester group in the polar position of a substituted cyclohexane has to stay in this position during hydrolysis and that the resulting crowded transition state (crowded due to steric interference of non-adjacent polar substituents) accounts for the relatively slow rate (reaction path A). On the other hand, it is also possible that prior to reaction the molecule is forced into a different conformation in which the ester group is now in the equatorial position (and some other, more bulky group in the polar position), and that the slower rate is due to the extra energy required to bring about the conformational transformation (reaction path B).

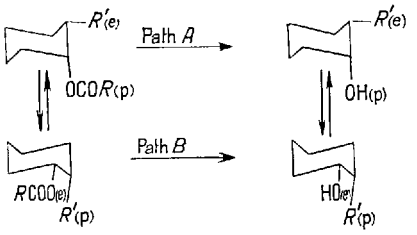
[1] M. Raymond-Hamet, C. r. Acad. Sci. *233*, 560 (1951).
[2] T. A. Henry, J. Chem. Soc. *1932*, 2759.
[3] M. Raymond-Hamet, C. r. Acad. Sci. *233*, 560 (1951).
[4] J. A. Goodson, J. Chem. Soc. *1932*, 2626.

[1] O. Hassel *et al.*, Acta Chem. Scand. *1*, 149, 929 (1947), and earlier papers.
[2] C. W. Beckett, K. S. Pitzer, and R. Spitzer, J. Amer. Chem. Soc. *69*, 2488 (1947).
[3] D. H. R. Barton, Exper. *6*, 316 (1950).

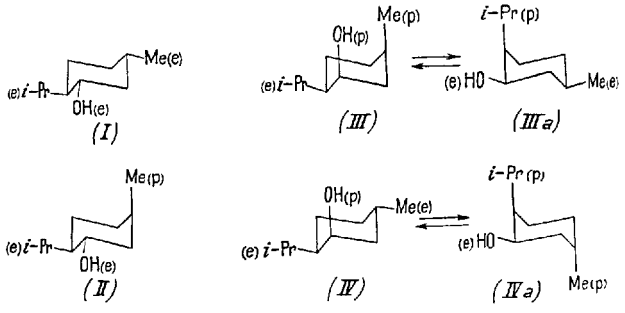
Stable Conformation					Conformation with OH-Group equatorial			
Group	Me	OH	*i*-Pr	*R**	Me	OH	*i*-Pr	*R**
Menthol (I)	e	e	e	1	e	e	e	1
Isomenthol (II)	p	e	e	2	p	q	e	2
Neoisomenthol (III) . . .	p	p	e	4	e	e	p	3 (IIIa)
Neomenthol (IV)	e	p	e	3	p	e	p	4 (IVa)

* *R* Expected order of reactivity.

Fortunately data are already in the literature to permit one to distinguish between these two possibilities.



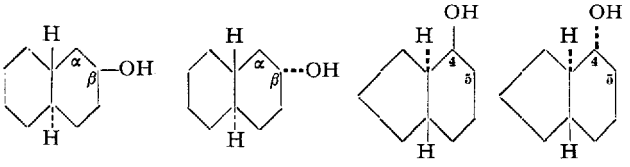
READ and GRUBB[1] have measured the relative rates of esterification of the menthols with *p*-nitrobenzoyl chloride by competitive reaction in pyridine at 25°C and found them to be 16·5 for menthol (I), 12·3 for *iso*menthol (II), 3·1 for neo*iso*menthol (III) and 1·0 for neomenthol (IV).



Presumably, the stable conformations of these molecules are such that the most bulky group (*iso*propyl) occupies an equatorial position[2], as shown in the Table at the top.

BARTON[3] has already pointed out that compounds having the hydroxyl group in the equatorial position in their most stable conformations are esterified faster than those having it in the polar position. Thus (I) and (II) are esterified faster than (III) and (IV). (II), which has a nonreactive substituent in the polar position reacts more slowly than (I) which has only equatorial substituents. However, if neo*iso*menthol and neomenthol reacted in their stable conformations—(III) and (IV)—one would expect (IV) to be esterified more rapidly than (III). In (III) there is severe crowding of the polar hydroxyl group by the nonadjacent polar methyl group while in (IV) the crowding is much less severe. Yet neomenthol (IV) is esterified only at one-third the rate of neo*iso*menthol (III). This is reasonable only if the molecules are converted to the less stable conformations—(IIIa) and (IVa)—prior to esterification. In these conformations—(IIIa) and (IVa)—the hydroxyl group is in the less crowded equatorial position and therefore more easily accessible to the esterifying reagent. The change from (IV) to (IVa) will be less facile than that from (III) to (IIIa), since in the former *two* alkyl groups (methyl and isopropyl) have to be forced into the crowded polar positions, in the latter only *one* (*iso*propyl). Therefore, making the reasonable assumption that (IIIa) and (IVa) are of the same order of reactivity, (III) should be esterified more readily than (IV), which is in accordance with the experimental facts.

A similar way of looking at the facts is to say that at room temperature (III) is in equilibrium with a small amount of (IIIa) [and (IV) with (IVa)]. Since (IIIa) is esterified more readily than (III), more (III) is continuously converted to (IIIa) until all has reacted in that form. Energetically, the two pictures are equivalent.



On the basis of this interpretation one would predict that cyclohexanols bearing a polar hydroxyl group *in a rigid system* which cannot change conformation so as to place the hydroxyl group in the equatorial position should show much greater difference in esterification and saponification rates (compared with their epimers with equatorial hydroxyl groups) than the more mobile molecules discussed above. Such rigid molecules are the *trans*-decalols (V) and *trans*-hydrindanols (VI) as well as the steroid alcohols. Quantitative data from the steroid field are not available, but qualitative observations[1] point to a large difference in ease of esterification and hydrolysis between epimers. The decalols,

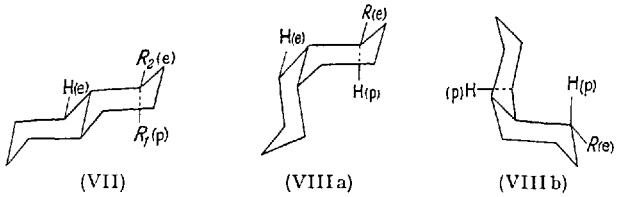
Compound	*k* 60°	*k* II/*k*I
trans-α-decalol, Isomer I (VIIa)	0·0382	
trans-α-decalol, Isomer II (VIIb)	0·725	19·0
trans-β-decalol, Isomer I	0·470	
trans-β-decalol, Isomer II	3·25	6·91
cis-*o*-methylcyclohexanol	0·0199	
trans-*o*-methylcyclohexanol	0·0352	1·77
cis-α-decalol, Isomer I (VIIIa)	0·438	
cis-α-decalol, Isomer II (VIIIb)	1·201	2·75

[1] J. READ and W. J. GRUBB, J. Chem. Soc. *1934*, 1779.
[2] W. HÜCKEL *et al.*, Ann. Chem. *533*, 128 (1937).
[3] D. H. R. BARTON, Exper. *6*, 316 (1950).

[1] L. F. FIESER, Exper. *6*, 312 (1950).

on the other hand, have been studied by HÜCKEL and co-workers[1]. Their results on the rate constants for hydrolysis of the decalyl phthalates in water at 60°C bear out the prediction that the difference between epimers in the *trans*-series are larger than in cyclohexane (see Table at the bottom of page 92).

The difference in hydrolysis rate between the epimeric *trans*-α-decalyl phthalates (VII a, b) would probably be even greater, were it not for steric interference of the *peri*-hydrogen atom with the ester-group where the latter is in the equatorial position. It is probably this interference of the *peri*-hydrogen atom which accounts for the sizable difference in saponification rates of the epimeric *cis*-α-decalyl phthalates (VIII a, b) despite the fact that both epimers in this series have the ester-group in the equatorial position.



(VII) (VIII a) (VIII b)

(a) $R_1 = o\text{-}C_6H_4$(COOH, COO—), $R_2 = H$; $R = o\text{—}C_6H_4$(COOH, COO—)

(b) $R_1 = H, R_2 = o\text{-}C_6H_4$(COOH, COO—)

Acknowledgement

The author is grateful to Professors H. C. BROWN and SAN-ICHIRO MIZUSHIMA for stimulating discussions regarding the stereochemistry of the menthols and decalols.

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Zusammenfassung

Aus der Veresterungsgeschwindigkeit der stereoisomeren Menthole mit *p*-Nitrobenzoylchlorid lässt sich schliessen, dass diese Moleküle in derjenigen Konstellation reagieren, in welcher die Oxygruppe eine äquatoriale Stellung einnimmt. In starren Molekülen (wie die *trans*-Dekalole), bei denen die Verschiebung einer Oxy- oder Estergruppe von der polaren in die äquatoriale Stellung nicht möglich ist, findet man besonders grosse Unterschiede in der Verseifungsgeschwindigkeit von epimeren Estern. Der Unterschied in der Verseifungsgeschwindigkeit von Estern der epimeren *cis*-α-Dekalole, die beide die Oxygruppe in der äquatorialen Stellung haben, lässt sich durch verschiedene sterische Hinderung durch eins der Wasserstoffatome in der *peri*-Stellung erklären.

[1] W. HÜCKEL *et al.*, Ann. Chem. *533*, 128 (1937).

Weitere Untersuchungen über Bakterienchemotaxine

In Arbeiten über chemotaktische Stoffe aus pathogenen Bakterien[1] konnte gezeigt werden, dass eine ganze Reihe von Bakterien, auf tierischem Plasmanährboden gehalten, Stoffe produzieren, die durch sterile Filtration abtrennbar *in vitro* starke chemotaktische Wirkung auf Leukozyten ausüben. Bei der Untersuchung gonadotroper Fraktionen aus sterilem Schwangernharn[1] ist es uns gelungen, ebenfalls Stoffe nachzuweisen, die eine stark anlockende Wirkung auf Leukozyten *in vitro* ausüben. Beide Stoffe haben zudem gemeinsam die Eigenschaft, dass sie thermostabil und nicht ultrafiltrierbar sind.

Zur weiteren Analyse der Natur solcher Chemotaxine untersuchten wir eine Reihe bedingt apathogener Keime. Die in Bouillon mit Ei während 72 h gezüchteten Bakterien wurden 3mal mit Tyrode gewaschen und in früher beschriebener Weise auf den Plasmanährboden neben die Leukozytenkulturen geimpft. Nach 24 h Bebrüten ergibt sich bei geeignetem Bakterienabstand ein Hinwandern der Leukozyten zur Bakterienkultur (Tab. I, Kol. 1, Chemotaktische Wirkung lebender Bakterien).

Die gewaschenen Bakterien wurden durch Hitze abgetötet, zentrifugiert und das Sediment mit einer Öse neben die Leukozytenkultur gebracht (Tab. I, Kol. 2, Chemotaktische Wirkung gewaschener, abgetöteter Bakterien). Werden gewisse chemotaktische Substanzen in Lösung gebracht, den Leukozytenkulturen überschichtet, so findet an Stelle der gerichteten Auswanderungsförderung eine allgemeine Vergrösserung des Auswanderungsareals statt, was damit gezeigt werden konnte, dass die gewaschenen, abgetöteten Bakterien in Tyrode aufgenommen wurden; eine Auswanderungsförderung konnte überall dort nachgewiesen werden, wo auch mit abgetöteten Bakterien eine Chemotaxis nachweisbar war (Tab. I, Kol. 3, Auswanderungswirkung gewaschener, hitzesterilisierter Bakterien). Weiterhin wurde der Bouillonnährboden auf Anwesenheit von auswanderungsfördernden Substanzen untersucht. Mit 24 h alten Bakterienkulturen wurden Nährböden beimpft, 72 h bebrütet und zentrifugiert. Die überstehende Bouillon wurde abpipettiert und im Dampftopf sterilisiert (Tab. I, Kol. 4, Auswanderungswirkung von Kulturlösungen).

Aus diesen Versuchen geht hervor, dass die meisten der untersuchten Bakterien auf tierischen Nährboden proliferierend eine chemotaktische Wirkung ausüben. Unter den auf Bouillon kultivierten gewaschenen und abgetöteten Keimen sind es jedoch mit Ausnahme von Diphtherie die gramnegativen, die thermostabile Chemotaxine enthalten. Diese thermostabilen Chemotaxine aus gramnegativen Keimen fördern in wässriger Lösung die Auswanderung, und es konnten in nennenswertem Ausmass nur in bakterienfreien Kulturlösungen gramnegativer Keime Stoffe gefunden werden, die Wirksamkeit besitzen.

In unsern chemischen Laboratorien wurden von BENZ aus den 72 h mit *Proteus vulg.* 0X19 bebrüteten und steril filtrierten Bouillons verschiedene Fällungen gemacht. Diese wurden entsprechend den zu ihrer Herstellung benötigten Bouillonmengen verdünnt und an der Leukozytenkultur mit der Wirkung des ursprünglichen Filtrates verglichen. Tabelle II zeigt die Wirkung der verschiedenen Fällungen auf das Auswanderungsareal der Leukozyten.

Alle diese Fällungen sind hitzebeständig und nicht dialysierbar. Bei Rohfällungen können durch wegdialysieren niedermolekularer Stoffe besser wirksame Präparate erzielt werden.

Aus diesen Versuchen lässt sich ableiten, dass sowohl grampositive wie auch gramnegative Keime auf tierischem Nährboden lebend in kurzer Zeit Chemotaxine produzieren. In Bouillon gezüchtet, enthalten und bilden

[1] R. MEIER, Helv. chim. Acta *24*, 134 E (1941).

[1] R. MEIER und B. SCHÄR, Exper. *7*, 308 (1951).