Tabelle I
Hydrazonbildung aus azetessigsaurem Natrium in wässeriger Lösung und bei Zusatz zu Blut

Einwaage in γ azetessigsaurem Natrium	*Als BTS direkt*		*Als BTS in % der eingewogen. Azetessigsäure*		BTS nach Kochen
	in wässeriger Lösung mg%	bei Zusatz zu Blut mg%	in wässeriger Lösung	bei Zusatz zu Blut	
100	0,65	0,72	6,5	7,2	–
200	1,26	1,60	6,3	8	–
300	2,04	2,20	6,8	7,3	–
400	2,76	3,10	6,9	7,7	–
500	3,10	3,30	6,2	6,6	–
600	3,60	5,00	6,0	8	–
700	4,54	5,40	6,4	7,7	–
800	5,66	6,10	7,1	7,7	–
900	6,32	6,50	7,0	7,2	–
1000	6,66	7,50	6,7	7,5	–

Tabelle II
Hydrazonbildung aus BTS, ohne und mit fünfminutigem Kochen, in wässeriger Lösung

BTS direkt mg%	BTS nach Kochen mg%	Verlust %
0,96	0,88	8,3
2,08	1,93	7,2
2,98	2,70	9,4
4,14	3,92	5,3
4,95	4,85	2,0

Tabelle III
Hydrazonbildung aus BTS, ohne und mit fünfminutigem Kochen, BTS-Zusatz zu Normalblut

	BTS direkt mg%	BTS nach Kochen mg%	Verlust %
Blut allein . .	1,2		
BTS-Zusatz, abzüglich Blutwert . .	1,1	1,1	0
	2,8	2,7	3,6
	5,4	5,1	5,6
	10,4	9,6	7,7

glauben also, mit dem sehr einfachen Verfahren des 5 Minuten dauernden Siedens des trichloressigsauren Filtrates vor dem Zusatz des 2,4-Dinitrophenylhydrazins einen zuverlässigen Weg zur Ausschaltung des Azetessigsäurefehlers bei der BTS-Bestimmung gefunden zu haben. Im folgenden wird der von uns angewendete genaue Untersuchungsgang beschrieben, der im Prinzip der Methode nach FRIEDEMANN und HAUGEN entspricht.

Methode

1 cm³ ungeronnenes Vollblut (Liqueminzusatz stört nicht, sehr genaue Abmessung erforderlich) wird sofort nach der Blutentnahme mit 5 cm³ 10prozentiger Trichloressigsäure zwecks Enteiweißung mit Glasstab gut durchmischt. Zentrifugieren. Anschließend wird das klare Zentrifugat in einem kleinen Reagensglas in kochendes Wasser verbracht und 5 Minuten am Sieden erhalten. Nach Abkühlung werden 3 cm³ des Zentrifugates mit 1 cm³ einer 0,1prozentigen 2,4-Dinitrophenylhydrazinlösung (das Hydrazin wird mit 2 *n* HCl gelöst) im kleinen Schütteltrichter versetzt und 5 Minuten reagieren gelassen (Zeit genau einhalten!). Anschließend Zugabe von 3 cm³ reinstem Toluol. 1 Minute kräftig durchschütteln, stehenlassen bis zur Trennung von Toluolextrakt und wässeriger Lösung. Verwerfung der wässerigen Phase. Dem Toluolextrakt werden 6 cm³ 10prozentige Natriumkarbonatlösung zugesetzt, dann wird wiederum 45 Sekunden kräftig geschüttelt. Die Trennung der Phasen wird abgewartet, dann erfolgt Filtrierung der wässerigen Phase in ein Reagensröhrchen. 3 cm³ des Filtrates werden mit 3 cm³ Natriumkarbonatlösung in einem 50-cm³-Erlenmeyerkölbchen verdünnt. Dieser Lösung werden vor der photometrischen Ablesung 5 cm³ 2*n* Natronlauge zugesetzt, wodurch Rotfärbung auftritt. Nach 5 Minuten Stehenlassen wird photometriert (am Lumetron Blau-Grün-Filter Nr. 490 benützen, am Pulfrich-Photometer Filter S 47).

Es ist notwendig, vor Beginn der BTS-Bestimmung im Blut eine Eichkurve mit reinstem Natriumpyruvat oder reinster, frisch destillierter BTS aufzunehmen. Die Lösungen bei der Bestimmung der Eichkurve werden ebenso behandelt wie das Blut.

S. MARKEES

Wissenschaftliches Laboratorium der Firma Hoffmann-La Roche, Basel, den 21. April 1951.

Summary

In the presence of aceto acetic acid the evaluation of pyruvic acid with 2,4-dinitrophenylhydrazin is disturbed because 6–8% of aceto acetic acid are determined as coloured hydrazon with the usual method. The elimination of aceto acetic acid can be obtained by putting the filtrate after deproteinization with trichloracetic acid into boiling water during 5 minutes. Less than 10% of pyruvic acid are destroyed by this procedure.

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

The Relative Stability of Stereoisomeric Forms of Fused Ring Systems

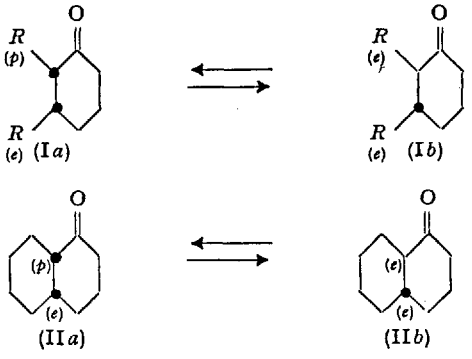
By WILLIAM S. JOHNSON[1]

The method of conformational analysis as applied so fruitfully to the steroids by BARTON[2] can be extended to rationalize the relative stability of certain fused ring systems. The premise that the cyclohexane ring is significantly more stable in the chair than the boat form, and that substituents on the ring are more stable in the equatorial (*e*) than the polar (*p*) conformation is now well accepted[1]. As has already been pointed out[1], in 1,2-disubstituted cyclohexanes it is possible for both substituents to be equatorial only when they have the trans configuration, while with the substituents cis it is necessary that one of them assume a polar conformation;

[1] Dep. of Chemistry, University of Wisconsin, Madison, Wisc., U.S.A.
[2] D. H. R. BARTON, Experientia *6*, 312 (1950).

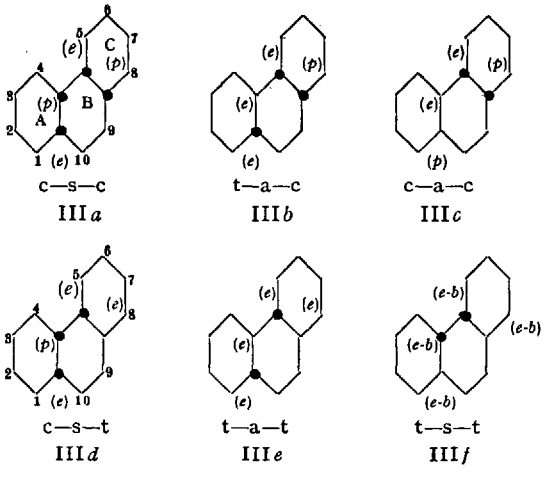
[1] For a summary of evidence see ref. 2.

hence, the trans form is generally more stable than the cis. It is obvious, then, that in cases where the two forms are interconvertible as with 2,3-disubstituted cyclohexanones I*a* and I*b*, which can be isomerized with base via the enolizable methyne hydrogen, it may be expected that the equilibrium will lie in favor of the form I*b*, *R* groups trans[1,2].



The situation is essentially the same when the two *R* groups represent a fused cyclohexane ring, and thus cis-α-decalone (II*a*) in which the cyclohexanone ring has one equatorial and one polar substituent, is isomerized to the trans isomer II*b* with two equatorial substituents[3]. Similar relative stability is found also in the corresponding hydrocarbons, where it has been shown independently that trans- is more stable than cis-decalin by conversion of the latter into the former with aluminium bromide or chloride[4].

The brilliant experiments of LINSTEAD and his collaborators[5] in the perhydrophenanthrene series where configurations have been established unequivocally provide a basis for testing the application of the foregoing principles to polycyclic compounds. The demonstration that the cis-syn-cis form (III*a* keto group at C_9) of 9-ketoperhydrophenanthrene is isomerized to and is consequently less stable than the cis-syn-trans form (III*d*, keto group at C_9)[4] is expected, since the terminal rings *A* and *C* are fused to the central ring *B* by 2(*e*) and 2(*p*) bonds in the former as compared with 3(*e*) and 1(*p*) bonds in the latter isomer. Similarly it has been shown[5] that the trans-anti-cis ketone (III*b*, keto group at C_9) with 3(*e*) and 1(*p*) linking bonds on ring *B* is isomerized to the more stable trans-anti-trans ketone (III*e*, keto group at C_9) with 4(*e*) bonds. While the two examples cited above are strictly analogous to the case of α-decalone, it has been shown that trans-ring fusion is not always more stable than cis. Thus LINSTEAD and WHETSTONE[7] found that the cis-syn-trans ketone III*d* (keto group at C_{10}) could not be isomerized to the trans-syn-trans form (III*f*, keto group at C_{10}), which is therefore concluded to be the less stable isomer. Conformational analysis with all rings assumed in the chair form gives 3(*e*) and 1(*p*) linking bonds for the former isomer and 2(*e*) and 2(*p*) bonds for the latter. From molecular models it appears, however, that the trans-syn-trans isomer (III*f*) cannot exist with the central ring *B* in the chair conformation, as it is impossible on steric grounds to have a ring fused in the trans configuration through two vicinal (*p*) bonds, since they lie perpendicularly on opposite sides of the ring. As a consequence this isomer would be expected to assume a chair-boat-chair arrangement with the end rings fused to ring *B* by four equatorial-boat bonds, i. e., 4(*e*—*b*). The greater stability of the cis-syn-trans isomer, hence becomes an experimental demonstration of the premise that the chair form is more stable than the boat comformation of cyclohexane. This evidently can be the case even when the chair form accommodates 1(*p*) substituent, i. e., a 3(*e*)—1(*p*) is more stable than a 4(*e*—*b*) arrangement.



On the basis of the foregoing, the following rules may be evolved[1]:

(1) *In saturated fused six-membered ring systems with all rings assumed to have the chair conformation those configurations with the larger number of (e) bonds at the point of ring fusion will be the more stable, the number of (e) and (p) bonds being determined as substituents of the most highly substituted rings and that alternative assignment being chosen which gives the greater number of (e) conformations.*

(2) *Since 2 meta (p) substituents (as in formula III a) show steric interference[2], such a form is less stable than one with 2 para (p) substituents (like III c).*

(3) *In configurations (like III f) where both alternative assignments lead to attachment of a single ring by 2 or-*

[1] The stereochemical method of representing formulas employed here is that of Linstead [see R. P. LINSTEAD, Chemistry and Industry *56*, 510 (1937), and R. P. LINSTEAD, W. E. DOERING, S. B. DAVIS, P. LEVINE, and R. WHETSTONE, J. Amer. Chem. Soc. *64*, 1985 (1942)], in which hydrogen atoms above the plane of the ring are represented by dots.

[2] This argument applies as well to 2,5-disubstituted cyclohexanones. With the 2,4- and 2,6-compounds, however, the trans forms would be expected to be less stable and hence isomerized to the cis configuration in which both groups can assume (*e*) conformations.

[3] W. HÜCKEL and E. BRINKMANN, Ann. Chem. *441*, 21 (1925); W. HÜCKEL, R. DANNEEL, A. GROSS, and H. NAAB, Ann. Chem. *502*, 99 (1933).

[4] N. D. ZELINSKY and M. B. TUROWA-POLLAK, Ber. Deutsch. Chem. Ges. *62*, 1658 (1929); *65*, 1299 (1932).

[5] R. P. LINSTEAD, W. E. DOERING, S. B. DAVIS, P. LEVINE, and R. WHETSTONE, J. Amer. Chem. Soc. *64*, 1985 (1942) *et seq.*

[6] R. P. LINSTEAD, R. R. WHETSTONE, and P. LEVINE, J. Amer. Chem. Soc. *64*, 2014 (1942).

[7] R. P. LINSTEAD and R. R. WHETSTONE, J. Chem. Soc., *1950*, 1428.

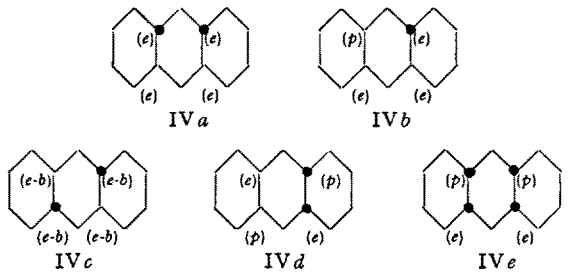
[1] These rules presumably apply to saturated systems with six or larger (medium sized) rings, but not to five and smaller rings which, because of internal steric factors tend toward coplanarity and hence favor cis- locking of the rings. Thus cis is somewhat more stable than trans hydrindane, and as the size of one ring is further reduced, the cis-configuration should become increasingly more stable with the cyclohexane ring tending toward the boat conformation. In the extreme case of a three- fused to a six-membered ring, the trans isomer would be so unstable that it might not be expected to exist.

In strainless fused ring systems where the bridgehead atoms are not connected together, conformational analysis may be applicable with appropriate modifications as, for example, in the case of 1,3-disubstituted cyclohexanes (cf. ref. 2, p. 316, left column).

[2] For a summary of evidence see ref. 2, p. 315.

tho(p) bonds, the ring involved may be forced into the boat conformation.

According to the above rules the six perhydrophenanthrenes have the following order of stability: III*e* [4(*e*)] > III*b* and III*d* [3(*e*), 1(*p*)] > III*c* [2(*e*), 2(*p*)] > III*a* [2(*e*), 2 meta(*p*)]; and III*f* [4(*e*–*b*)] < III*b* and III*d*.



Similar analysis of the perhydroanthracenes leads to the following order of stability: IV*a* [4(*e*)] > IV*b* [3(*e*), 1(*p*)] > IV*d* [2(*e*), 2(*p*)] > IV*e* [2(*e*), 2 meta(*p*)]; and IV*c* [4(*e*–*b*)] < IV*b*. Therefore that isomer, m. p. 90°, which is formed from IV*e* or IV*b* by the action of aluminium chloride[1] probably corresponds to IV*a*, and the form IV*c* which was suggested by COOK, MCGINNIS and MITCHELL[1] as a possible, although less probable, alternative because it also contains two trans fused ring systems, can be excluded. The boat-boat-boat conformation of IV*e* (m. p. 61°) suggested by these authors might indeed be less stable than the chair-chair-chair form in spite of the 2 meta (*p*) substituents required by the latter.

Zusammenfassung

Ausgehend von neueren Anschauungen über die Stabilität verschiedener Konstellationen (conformations) monozyklischer Ringverbindungen wurden die Verhältnisse bei perhydrierten polyzyklischen Verbindungen diskutiert. Daraus konnten gewisse Regeln über die relative Stabilität der einzelnen Stereoisomeren abgeleitet werden.

[1] J. W. COOK, N. A. MCGINNIS, and S. MITCHELL, J. Chem. Soc. *1944*, 286.

Effect of Urethane on the Incorporation of C^{14} into Animal Tissue

By G. HEVESY, R. RUYSSEN, and M. L. BEECKMANS[1]

From previous experiments it is known that administration of urethane enhances the incorporation of C^{14} into most tissue fractions of the mouse after injection of labeled acetate[2]. The present paper deals with the effect of urethane on the incorporation of C^{14} into phosphatides, cholesterol, and other tissue fractions of various organs of the mouse.

Experimental

In each experiment two groups of 10 to 15 adult mice of nearly equal weight (20 g) were used. Into all animals was injected intraperitoneally 0·2 ml of sodium chloride solution containing about 0·2 mg sodium acetate labeled in the carboxyl group[3].

After five minutes the urethane group was injected intraperitoneally with 0·15 ml of a 20 % aqueous urethane solution, while the control group received 0·15 ml of a physiological salt solution. The animals were killed at different times.

The same organs from each group were combined and frozen in solid CO_2. A small fraction was directly dried at 70°C and measured as total tissue.

The ground organs were extracted with a boiling mixture of ether-alcohol $^1/_3$ for 3 hours. The residue was washed repeatedly with water, then treated twice for 10 minutes at 90°C with a 5 % aqueous solution of trichloroacetic acid, the proteins being thoroughly rinsed with water after each treatment; they were then dried by washing with alcohol and ether.

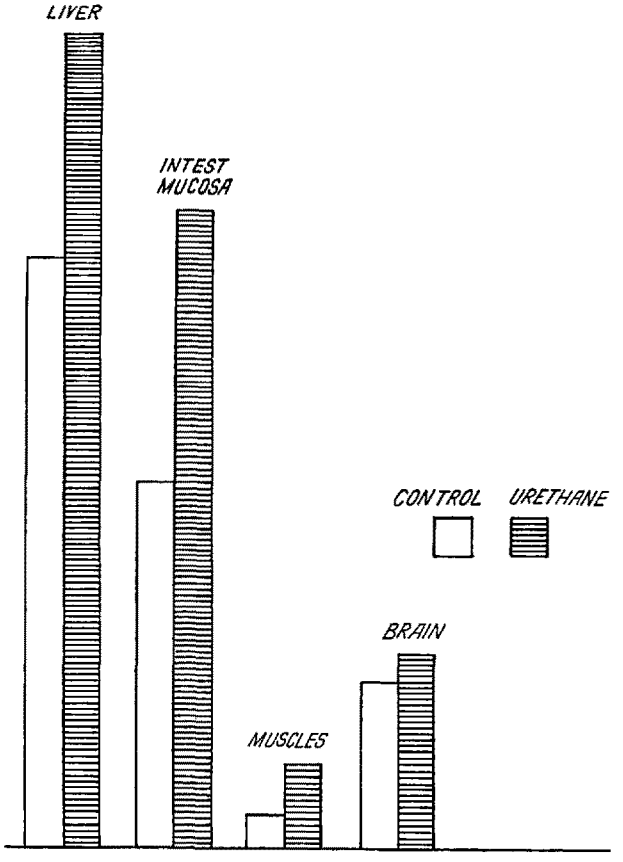


Fig. 1. – Effect of urethane on the incorporation of C^{14} into tissue phosphatides. Mice killed 100 minutes after injection of $NaCH_3C^{14}OO$.

The ether-alcohol solution was evaporated and the residue extracted with petroleum-ether. After evaporation of the petroleum-ether the total fats remained. They were purified from urea and other impurities according to the procedure proposed by FOLCH and VAN SLYKE[1]. This procedure involves the loss of some of the phosphatides.

In order to obtain the phosphatides, the total fats were dissolved in petroleum-ether and precipitated with twice the amount of cold acetone and 5 drops of an alcoholic solution of 4·5 % $MgCl_2$, precipitation being completed when the preparation had stood for 2 hours in the refrigerator. The phosphatides were centrifuged. The precipitate, dissolved in petroleum-ether, was precipitated again in the same way. After centrifugation the precipitate was dissolved in petroleum-ether and the $MgCl_2$ removed by repeatedly washing with distilled water. After evaporation of the petroleum-ether the phosphatides remained.

[1] Institute for Research in Organic Chemistry, University of Stockholm, and Pharmaceutical Institute, University of Gent.

[2] G. HEVESY, Nature *164*, 1007 (1949).

[3] We are much indebted to Dr. LOOS for preparing the labeled acetate.

[1] J. FOLCH and D. D. VAN SLYKE, Proc. Soc. Exp. Biol. and Med. *41*, 514 (1939).