due to the presence of alkyl homologs of chrysene. This was substantiated by the proton magnetic resonance spectrum of the material present in the mother liquors from recrystallization of the crude chrysene, which showed signals in the region characteristic of aromatic methyl groups. Analysis of material CH: found, C, 94.50; H, 5.73. Calculated for chrysene, $C_{18}H_{12}$, C, 94.70; H, 5.30; mol. wt., 228.

Rechromatography of CH on alumina gave on elution with hexane-benzene (4:1) a colorless compound which crystallized from hexane as shining, colorless leaflets, m.p. 256–257°, which did not depress the melting point of authentic chrysene (m.p. 257–259°). The mass spectrum of this material was essentially identical with that of authentic chrysene: it showed a molecular ion peak at m/e 228, and the higher peaks, at intervals of 14 mass units, present in the original material (CH), were absent. The UV-spectrum (in 95% ethanol) was identical with that of chrysene: λ_{max} 220 nm (log ε 4.58); 241 (4.35); 258.5 (4.93); 268 (5.17); 282.5 (4.11); 294 (4.08); 306 (4.11); 320 (4.11); 343 (2.65); 351 (2.20); and 361 (2.71).

Fractions 10–16, also eluted with hexane-benzene, afforded a colorless solid which crystallized from chloroform-hexane as colorless platelets, m.p. 235–250°. Rechromatography of this substance on alumina and elution with hexane-benzene (10:1) gave a colorless solid which, after recrystallization from hexane, had m.p. 250–262°. The mass spectrum showed that it was a complex mixture with a base peak at m/e 256. Although the 'cracking pattern' of chrysene could be discerned, the chrysene M^+ peak at 228 was only 25% of the base peak, and the peak at 242 was 38% of the base peak.

X-ray powder diagrams. The X-ray powder diagrams of the picene and chrysene from the mineral were compared with those of the authentic specimens, and showed complete identity, thus providing further confirmation of their identities.

Discussion. The origins of coronene, the organic constituent of pendletonite[9], picene and chrysene as massive constituents of these organic minerals, are not known with certainty. MALEEVA[10] has suggested that Transcarpathian curtisite, which is accompanied by bitumens, may be a product of the distillation (sic) of organic substances contained in the sedimentary stratum. Since curtisite is present in veins of probably magmatic origin in sedimentary and metamorphic rocks, it is possible that these stable, polycyclic aromatic hydrocarbons have been derived by pyrolytic transformations of organic matter and deposited, by distillation or from solution, in the formations in which they are now found. It is to be recalled that chrysene and methylated picenes (but not coronene) are among the products formed in the high temperature aromatization of steroidal and triterpenoid compounds. It is worthy of note, however, that the Skaggs Springs deposits are not associated with bituminous or petroleum deposits.

Zusammenfassung. Das massive organische Mineral Curtisite (Idrialite) besteht aus Picen, Chrysen und, wie aus den Massenspektren der rohen Proben zu entnehmen ist, deren Methyl-homologen.

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[9] J. MURDOCH and T. A. GEISSMAN, Am. Miner., in press.
[10] T. P. MALEEVA, Mineralog. Sb. L'vov *8*, 85 (1954); Chem. Abstr. *52*, 989 (1958).

1,2-Methylen-19-nor-17α-acetoxy-progesteron

Die aufgefundenen starken hormonellen[1], proteinanabolen[2] und antiandrogenen[3] Wirkungen in der Reihe der 1,2α-Methylen-Steroide liessen uns die Synthese des 1,2α-Methylen-19-nor-17α-acetoxy-progesterons interessant erscheinen.

Das Fehlen der dirigierenden C_{10}-Methylgruppe lässt eine strukturelle Zuordnung der nach der COREY[4]-Methylenierung von Δ^1-3-Keto-A/B-cis- bzw. A/B-trans-Steroiden zu erwartenden 1,2-Methylen-Verbindungen in Analogie zu C_{10}-Methyl-Steroiden[5,6], nicht ohne weiteres zu.

Deshalb synthetisierten wir sowohl das 1,2α-Methylen-19-nor-17α-acetoxy-progesteron als auch das 1,2β-Methylen-Isomere, um aus deren physikalischen Daten Strukturanhaltspunkte zu finden.

Aus Δ^5-Pregnen-3β,17α,19-triol-20-on-17-acetat[7] (1) entsteht durch Hydrierung (PtO_2/Methanol) das entsprechende 5α-Pregnan-Derivat (F. 231–233 °C) und daraus durch anschliessende Oppenauer-Oxydation das 5α-Pregnan-17α,19-diol-3,20-dion-17-acetat (2a) (F. 232,5-234,5 °C). Nach Acetylierung von (2a) zu (2b) (F. 152-153 °C) wird über Bromierung/Bromwasserstoffabspaltung die Δ^1-Doppelbindung zu (3b) (F. 157,5–159 °C, UV: ε_{230} = 9830) eingeführt.

Die 19-Acetoxy-Gruppe in (3b) wird mit dem alkalischen Dowex-Ionen-Austauscher verseift zum 19-Alkohol, (3a) (F. 263–266 °C) dessen Chromsäure/Pyridin-Oxydation den 19-Aldehyd (4) (F. 232–234 °C) ergibt. Durch Säurespaltung wird die C_{10}-Formylgruppe eliminiert zu (5b) (F. 181,5–184 °C, UV: ε_{230} = 11100). Nach alkalischer

[1] R. WIECHERT und F. NEUMANN, Arzneimittel-Forsch. *15*, 244 (1965).
[2] F. NEUMANN und R. WIECHERT, Arzneimittel-Forsch. *15*, 1168 (1965).
[3] F. NEUMANN, W. ELGER und R. v. BERSWORDT-WALLRABE, Dt. med. Wschr. *92*, 360 (1967).
[4] E. J. COREY und M. CHAYKOVSKY, J. Am. chem. Soc. *84*, 867 (1962).
[5] H.-G. LEHMANN, Dt. Bundespatent 1.183.500 (1962, Schering AG); G. W. KRAKOWER und H. A. VAN DINE, J. org. Chem. *31*, 3467 (1966).
[6] R. WIECHERT, O. ENGELFRIED, U. KERB, H. LAURENT, H. MUELLER und G. SCHULZ, Chem. Ber. *99*, 1118 (1966).
[7] A. BOWERS, U.S. Patent 3.065.228 (1962, Syntex S.A.).

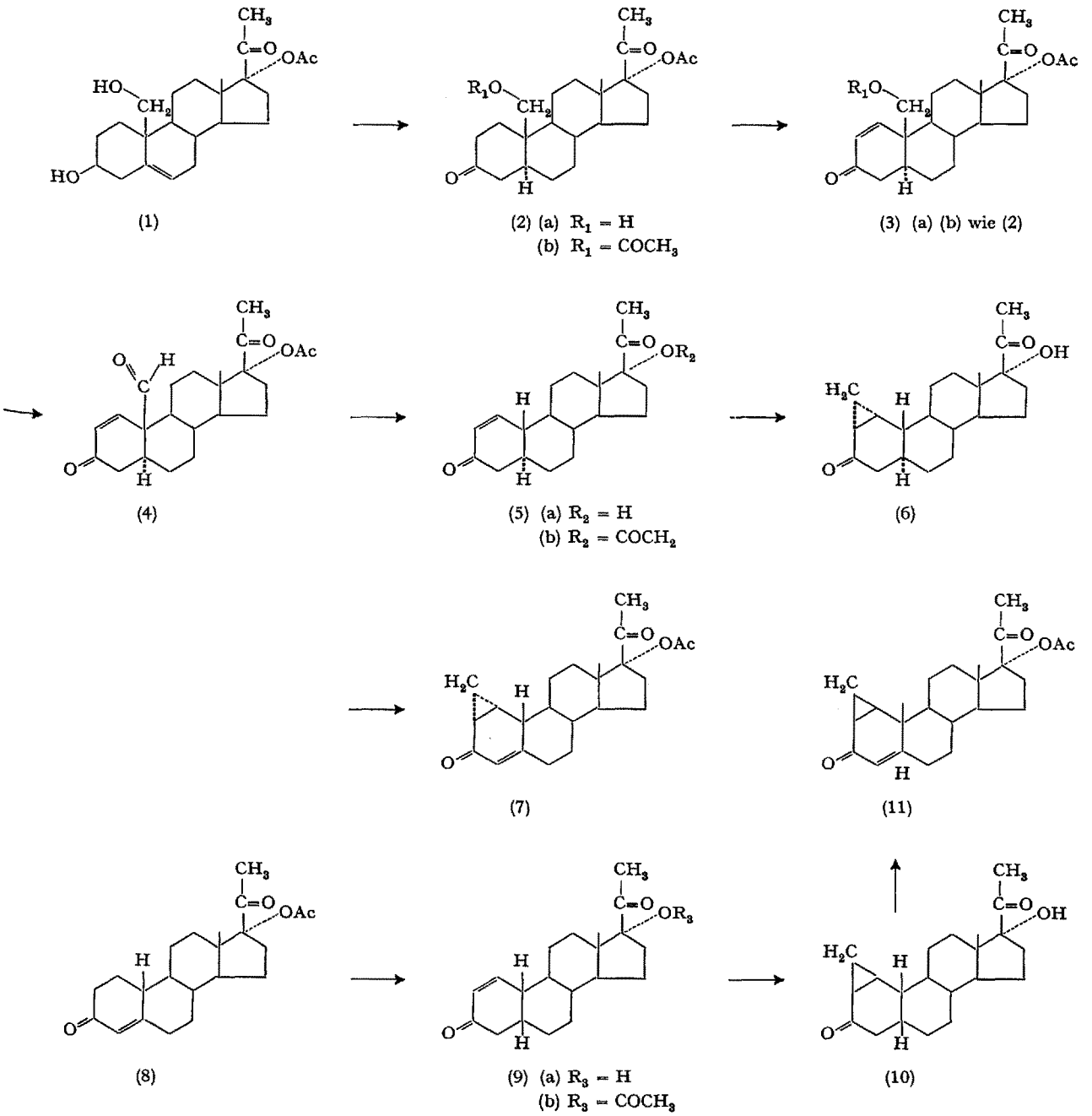
Verseifung von (5b) zu (5a) (F. 243–247,5 °C) wird dieses mit Dimethylsulfoxoniummethylid in 1,2-Stellung methyleniert zu (6) (F. 220,5–222,5 °C, UV: $\varepsilon_{208} = 3870$). Die Δ^4-Doppelbindung und das 17-Acetat wurde schliesslich ohne Zwischenreinigung durch Enolacetylierung, Bromierung und Bromwasserstoffabspaltung eingeführt.

Das Endprodukt (7) (F. 185,5–186 °C, aus Essigester, F. 222–223 °C, aus Isopropyläther, $[\alpha]_D^{25}$: +166,3 ($c = 0{,}80$ $CHCl_3$), UV: $\varepsilon_{241} = 13100$) hat im Nah-IR die typischen Dreiringbanden bei 6130/cm und 4520/cm, im NMR (TMS als interner Standard, Lösungsmittel $CDCl_3$) liegt das olefinische Proton am C_4 bei $\delta = 5{,}60$ ppm.

Die isomere 1,2-Methylen-Verbindung zu (7) wurde ausgehend vom 19-Nor-17α-acetoxy-progesteron (8)[8] dargestellt. Nach der Hydrierung (Pd/Methanol) von (8) mit anschliessender Bromierung und Dehydrobromierung wird die Δ^1-5β-Verbindung (9b) (F. 162,5–164 °C) isoliert. Nach Verseifung (9a) (F. 178,5–180 °C, UV: $\varepsilon_{231} = 9990$), Methylenierung (10) (F. 166–174 °C) und analogen Reaktionen wie bei (6 → 8) wird das methylenierte 19-Nor-17α-acetoxy-progesteron (11) (F. 212,5–214 °C, UV: $\varepsilon_{241} = 12000$, $\varepsilon_{220} = 8390$) erhalten. Das NMR-Signal des olefinischen Protons am C_4 liegt in (11) bei $\delta = 5{,}65$ ppm, ist also gegenüber (7) um 0,05 ppm entschirmt.

Die NMR-Daten und das für 1,2β-Methylen-Δ^4-3-Ketone[6] typische UV-Maximum bei 220 nm in (11) er-

[8] A. Zaffaroni, H. J. Ringold, G. Rosenkranz, F. Sondheimer, G. H. Thomas und C. Djerassi, J. Am. chem. Soc. *80*, 6110 (1958).



lauben die im Formelschema für (7) und (11) gegebenen Zuordnungen.

Der sterische Verlauf der COREY-Methylenierung von Δ^1-3-Keto-Steroiden der A/B-cis- und A/B-trans-Reihe ist also unabhängig von der angulären C_{10}-Methylgruppe.

Im Tierversuch wird an Kaninchen bei peroraler Applikation im Clauberg-Test die Aktivität des 19-Nor-17α-acetoxy-progesterons durch die Einführung der 1,2α-Methylengruppe stark erhöht und durch die 1,2β-Methylengruppe abgeschwächt.

Summary. The syntheses and structure-determinations of 1,22α- and 1,2β-methylene-19-nor-17α-acetoxy-progesterones described prove that there is no influence of the angular C_{10}-methylgroup on the steric course of the COUREY-methylenations of Δ^1-3-keto-steroids.

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Diketopiperazines from Fermentations: Metabolites, Artifacts, or Both

The frequent isolation in our laboratory of L-leucyl-L-proline anhydride (I) and, occasionally, other diketopiperazines from extracts of fermentations designed either for modification of steroids or preparation of antibiotics led us to consider the source of this material. In our hands, at this stage, the isolation of I was fortuitous in every instance and was usually due to similarity in solubility properties with the desired product.

Numerous authors have reported the isolation of I and other diketopiperazines from microbiological sources[1-12]. Studies in some laboratories[1,7,10,11] claim that I cannot be isolated from unfermented culture media, and these reports, combined with the reported isolation of I from silkworm pupae[13] and adrenal cortex extracts[14,15] made it appear that I is a common metabolite. Our frequent encounters with I initially lent some support to this view. However, when we examined several typical unfermented media, we were always able to isolate I by extraction with ethyl acetate and chromatography over alumina. The isolated crystalline product is identical in melting point, optical rotation, analytical data, IR- and nmr-spectra with authentic I and, further, gave leucine and proline upon hydrolysis. Minor amounts of other diketopiperazines were encountered occasionally.

Looking further into the matter, we have isolated 181 mg of I/kg of peptone, 227 mg/kg of corn steep liquor and a smaller amount from lactalbumin hydrolyzate. TAMURA et al.[16] have also found diketopiperazines in unfermented peptone and their data show about 1% of I and smaller amounts of other diketopiperazines. In fermentations wherein we encountered I, the above complex nitrogen sources were invariably present, and always in sufficient quantity to explain the presence of I in the extracts.

In view of these data, it is not surprising that relatively large quantities of diketopiperazines, especially I, are frequently isolated from fermentation mixtures. The occurrence of these substances may often reflect the previous history of the media constituents, particularly in regard to hydrolysis of protein and subsequent exposure of the products to heat[17].

As microbes are quite capable of protein hydrolysis it is also likely, indeed probable, that a certain proportion of the diketopiperazines present may arise during the fermentation to replace or supplement the quantity supplied in the medium. This may rationalize the conflicting claims on the subject. Nevertheless, we feel that the apparent production of simple diketopiperazines in fermentation processes should be viewed with some measure of restraint and invariably be accompanied by careful control experiments to eliminate the strong possibility of mistaking an artifact for a valid metabolite. This precaution is especially important as a number of complex or more highly elaborated diketopiperazine derivatives (e.g. sporidesmins-gliotoxins etc.) have been isolated from fermentations and their biological properties have made their biosynthetic origin a subject of considerable importance[12,18-21].

Zusammenfassung. Es wird gezeigt, dass Diketopiperazine, insbesondere L-Leucyl-L-prolin-anhydrid, eher aus dem Medium stammende Artefakte als echte mikrobielle Stoffwechselprodukte sind.

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[1] J. L. JOHNSON, W. G. JACKSON and T. EBLE, J. Am. chem. Soc. *73*, 2947 (1951).
[2] L. ETTLINGER, E. GÄUMANN, R. HÜTTER, W. KELLER-SCHIERLEIN, F. KRADOLFER, Z. NEIPP, V. PRELOG, P. REUSSER and H. ZÄHNER, Mh. Chem. *88*, 989 (1957).
[3] S. H. EPPSTEIN, D. H. PETERSON, H. M. LEIGH, H. C. MURRAY, A. WEINTRAUB, L. M. REINECKE and P. D. MEISTER, J. Am. chem. Soc. *75*, 421 (1953).
[4] T. KOSUGE and H. KAMIYA, Nature *188*, 1112 (1960).
[5] T. KOSUGE and H. KAMIYA, Chem. pharm. Bull., Tokyo *10*, 154 (1962).
[6] N. INAGAKI, Chem. pharm. Bull., Tokyo *10*, 152 (1962).
[7] G. LUEDEMANN, W. CHARNEY, A. WOYCIESJES, E. PETTERSEN, W. D. PECKHAM, M. J. GENTLES, H. MARSHALL and H. L. HERZOG, J. org. Chem. *26*, 4128 (1961).
[8] Y. KODAIRA, Agric. biol. Chem. Japan *25*, 261 (1961).
[9] Y. KOAZE, Agric. biol. Chem. Japan *24*, 530 (1960).
[10] Y.-S. CHEN, Agric. biol. Chem. Japan *24*, 372 (1960).
[11] Y. KOAZE, Agric. biol. Chem. Japan *24*, 449 (1960).
[12] C. KELLEY and R. BROWN, Experientia *22*, 721 (1966).
[13] A. BUTENANDT, P. KARLSON and W. ZILLIG, Hoppe-Seyler's Z. physiol. Chem. *288*, 279 (1951).
[14] O. WINTERSTEINER and J. J. PFIFFNER, J. biol. chem. *111*, 599 (1935).
[15] M. H. KUIZENGA, J. W. NELSON, S. C. LYSTER and D. J. INGLE, J. biol. Chem. *160*, 15 (1945).
[16] S. TAMURA, A. SUZUKI, Y. AOKI and N. OTAKE, Agric. biol. Chem. Japan *28*, 650 (1964).
[17] E. ABDERHALDEN, Hoppe-Seyler's Z. physiol. Chem. *128*, 119 (1923).
[18] M. W. MILLER, *The Pfizer Handbook of Microbial Metabolites* (McGraw-Hill Book Co., Inc., New York 1961), p. 460, 496.
[19] D. BREWER, D. E. HANNAH and A. TAYLOR, Can. J. Microbiol. *12*, 1187 (1966).
[20] G. LOWE, A. TAYLOR and L. C. VINING, J. chem. Soc. (C) *1966*, 1799.
[21] J. W. RONALDSON, A. TAYLOR, E. P. White and R. J. ABRAHAM, J. chem. Soc. (C) *1963*, 3172.