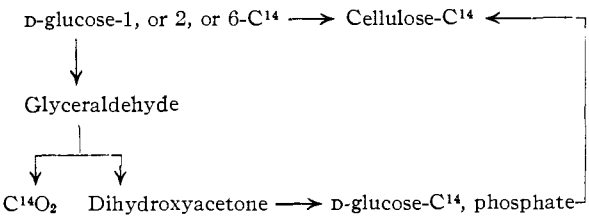
the six carbon atoms is labeled and on the time of adding the radioactive sugar. Thus, the mechanisms of cellulose polymerization is complex. At least two major mechanisms appear from these studies, (1) direct polymerization, possibly involving phosphorylation and (2) cleavage of the hexose and resynthesis of hexose phosphates from trioses such as glycerol. Hexose phosphates[12] have been isolated and identified as intermediates in the biosynthesis of cellulose from D-glucose, thus the reason for suggesting these products as possible energy sources in the major mechanism of polymerization.

To summarize the research, a schematic representation of the processes of cellulose formation may be shown as



A cell-free enzyme system has been isolated from *A. xylinum* capable of producing cellulose-C^{14}.[13] D-Glucose-1-C^{14} was polymerized to cellulose and the hydrolyzate (D-glucose) analyzed. Ninety-six per cent. of the label was found in position one of the cellulose molecule.

(12) Unpublished data.

(13) G. A. Greathouse, THIS JOURNAL, **79**, 4503 (1957).

GAINESVILLE, FLA.

[JOINT CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE STATE UNIVERSITY AND THE INSTITUTO DE QUIMICA AGRICOLA, MINISTERIO DA AGRICULTURA, RIO DE JANEIRO]

The Chemistry of Rosewood. Isolation and Structure of Anibine and 4-Methoxyparacotoin[1]

BY WALTER B. MORS,[2a,3] OTTO RICHARD GOTTLIEB[2b,3] AND CARL DJERASSI

RECEIVED APRIL 12, 1957

From the wood of the South American rosewood trees (genus *Aniba*) there has been isolated a new alkaloid, $C_{11}H_9NO_3$, which has been named anibine. By various degradations, in particular by alkaline cleavage, it was shown that anibine is 4-methoxy-6-(3′-pyridyl)-α-pyrone (IV). A neutral companion substance of anibine, when subjected to similar reactions, was proved to be 4-methoxy-6-piperonyl-α-pyrone (X) (4-methoxyparacotoin) and attention is called to the structural similarity with the constituents of the closely related *Coto* barks.

The South American rosewood trees belong to the *Lauraceae* family, and the derived essential oil has long been an article of commerce.[4] The botanical identification has been confusing, and the trees were once believed to belong to the genus *Ocotea*.[5] It is now accepted that the *bois de rose* from French Guiana and adjacent areas is *Aniba rosaeodora* Ducke and the Brazilian *páu rosa* from the lower Amazon basin *Aniba Duckei* Kostermans (*A. rosaeodora* var. *amazonica* Ducke).[6,7] The main constituent of the rosewood essential oil is linalool, but a ready differentiation of the two species is even possible on chemical grounds since the essential oil from French Guiana is strongly levorotatory while the Brazilian one is only slightly so or even dextrorotatory.[4]

Aside from the essential oil, no chemical work seems to have been done with these plants, and a detailed examination of the composition of these *Aniba* species was undertaken by one of us (O.R.G.) The Brazilian *Aniba Duckei* Kostermans represented commercial material from Manaus (State of Amazonas) while a sample of *Aniba rosaeodora* Ducke came from an isolated tree from the region of the Amaparí river (Territory of Amapá, Brazil).[8] A number of products were isolated, and the present paper is concerned with the alkaloid and one of the neutral constituents, which were found in both *Aniba* species.

Extraction of the rosewood sawdust with benzene followed by removal of basic material with hydrochloric acid and basification yielded a single, crystalline alkaloid, m.p. 179–180°, in *ca.* 2.6% yield. Its empirical formula, $C_{11}H_9NO_3$, immediately indicated that it must differ considerably from the few alkaloids (isoquinoline types) hitherto encountered among the *Lauraceae*,[5,9] and we have named the substance "anibine." Functional group analysis demonstrated the presence of one methoxyl group and the absence of N-methyl or C-methyl groups. Anibine contained no active hydrogen atom and was optically inactive over the range 700–400 mμ. The alkaloid, though forming a crystalline methiodide, hydrochloride and picrate, was weakly basic and in fact could be removed from hydrochloric acid solution by continuous extraction with chloroform. The nitrogen atom was, therefore, assumed to be part of a heterocyclic ring which was not inconsistent with the ultraviolet

(1) This article should be considered paper XVIII in the Wayne series "Alkaloid Studies"; for preceding article see THIS JOURNAL, **79**, 2203 (1957).

(2) (a) Rockefeller Foundation Fellow at Wayne State University, 1956–1957. (b) Acknowledgment is due to the Conselho Nacional de Pesquisas, Brazil, for financial aid.

(3) Instituto de Quimica Agricola, Rio de Janeiro, Brazil.

(4) E. Guenther, "The Essential Oils," Vol. IV, Van Nostrand Co., Inc., New York, N. Y., 1950, p. 191.

(5) See C. Wehmer, "Die Pflanzenstoffe," Vol. I, Gustav Fischer, Jena, 1929, pp. 364–365. References to earlier chemical investigations of the *Lauraceae* can be found on pp. 350–373.

(6) A. Ducke, *Arq. Jard. Bot. Rio de Janeiro*, **5**, 109 (1930).

(7) A. J. G. H. Kostermans, *Rec. trav. bot. neerl.*, **35**, 918 (1938).

(8) The botanical identification was carried out by Dr. Arthur de Miranda Bastos (Botanical Garden, Rio de Janeiro) and confirmed by the rotation ($[\alpha]_D$ −16°) of its essential oil.

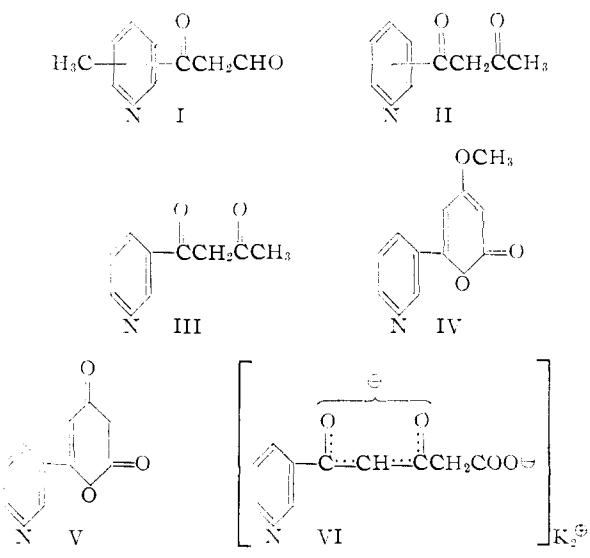
(9) See T. A. Henry, "The Plant Alkaloids," Blakiston, Philadelphia, 1949, p. 319.

absorption data (see Experimental). In accordance with the observed absence of active hydrogen, the infrared spectrum showed no bands corresponding to OH or NH absorption but exhibited intense bands at 5.77, 6.02 and 6.33 μ. The lability of anibine toward alkali suggested the presence of a lactone system (5.77 μ) which together with the methoxyl group would account for all three oxygen atoms. The 6.02 μ band could be assigned to an enol ether and this was proved subsequently to be correct. Catalytic hydrogenation (platinum oxide in acetic acid) resulted in the uptake of 6 moles of hydrogen which indicated that hydrogenolysis must have occurred,[10] although no pure product could be isolated.

An ethanolic alkaline solution of anibine turns yellow immediately, but the spectrum (λ_{max}^{KOH} 252, 282 and 381 mμ) changes upon long standing (λ_{max}^{KOH} 334 mμ), and the color is virtually discharged. When this reaction was carried out on a preparative scale, there was isolated an insoluble dipotassium salt ($C_{10}H_7K_2NO_4 \cdot H_2O$), which in contrast to anibine itself did not contain any methoxyl group. Acidification of an aqueous solution of the potassium salt resulted in decarboxylation—indicating the presence of a β-keto acid—and the isolation of an amphoteric crystalline solid, $C_9H_9NO_2$. The latter also could be obtained in somewhat poorer yield by boiling anibine with acid. The absence of a methoxyl group already was noted in the potassium salt precursor, but it was now observed that the cleavage product contained one C-methyl group, which was not present in the parent alkaloid. Consequently, this new C-methyl group must represent the point of attachment of the potential carboxyl group of anibine which was lost by decarboxylation during the acid and alkaline cleavage reactions.

The infrared spectrum of the $C_9H_9NO_2$ cleavage product was very instructive since it showed no definite OH absorption or significant carbonyl absorption below 6 μ. Rather, it exhibited a broad band in the 6.2 μ region typical of carboxylate or enolate anions, and the above-mentioned chemical and analytical data were only compatible with the latter. Catalytic hydrogenation resulted in the uptake of 5 moles of hydrogen, and since only 6 units of unsaturation are permissible in $C_9H_9NO_2$, the substance can only contain one ring, from which it follows that the tertiary nitrogen atom in a heterocyclic ring present in anibine must be represented by a pyridine ring. A $C_9H_9NO_2$ formulation containing one C-methyl group, a pyridine ring and a β-diketone enolate moiety can only be incorporated in partial formulas I or II. Of these, the former is rather unlikely since model Kuhn–Roth oxidations with various methylated pyridines showed that virtually no acetic acid was produced while the cleavage product yielded slightly over 100% of volatile acid. All three possible isomers of II have been described in the literature, and it was shown by direct comparison with a synthetic specimen that the $C_9H_9NO_2$ cleavage product of anibine was identical with the known[11] 1-(3′-pyridyl)-butane-1,3-dione (β-acetoacetylpyridine)-(III).[12]

With this information at hand, anibine can only be represented by 4-methoxy-6-(3′-pyridyl)-α-pyrone (VI). As stated above, the absence of a C-methyl group in anibine requires that the carboxyl group of the lactone be attached to the end of the side chain of III. Since anibine clearly did not contain a β-lactone ring, lactonization must have involved the oxygen atom at the δ-position, leading to structure V. Anibine, however, contains one methoxyl group which can only be incorporated into V as an enol ether grouping, thus resulting in expression IV for anibine,[13] which appears to be the first alkaloid containing an α-pyrone ring.



Crystallization of the neutral benzene-soluble fraction of rosewood furnished a colorless solid, m.p. 221–224°, possessing the empirical formula $C_{13}H_{10}O_5$. The substance possessed one methoxyl and no C-methyl group, exhibited no hydroxyl absorption but showed general spectral similarities to anibine. When boiled with alkali, there was isolated piperonylic acid and 3,4-methylenedioxyacetophenone (VII). On the other hand, when the reaction was conducted at room temperature with ethanolic potassium hydroxide as was the case with anibine (IV), there was isolated an insoluble potassium salt. Acidification led to an unstable β-keto acid, m.p. 125–130°, shown to have structure VIII, since it was decarboxylated readily upon heating or attempted recrystallization to the previously unknown 1-piperonyl-butane-1,3-dione (IX). This compound was identified by comparison with a synthetic specimen prepared from methyl piperonylate and acetone. By employing precisely the same arguments outlined above for ani-

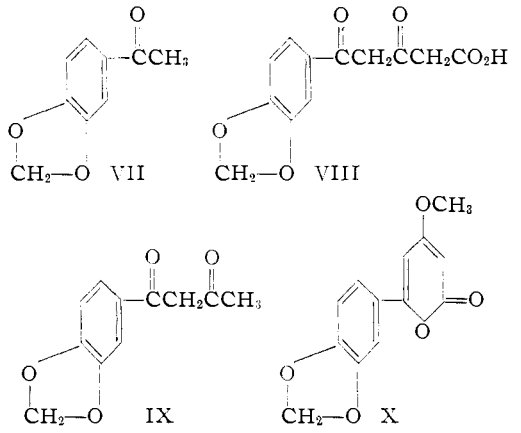
(10) The empirical formula of anibine permits eight double bonds or rings. The presence of a lactone ring and of a heterocyclic ring in the parent alkaloid would allow only for the uptake of 5 moles of hydrogen unless hydrogenolysis occurred as well.

(11) A. Ferenczy, *Monatsh.*, **18**, 673 (1897).

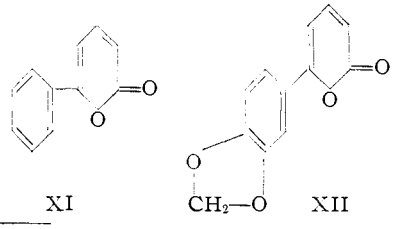
(12) Subsequently, nicotinic acid was isolated in poor yield in one alkaline cleavage reaction and by nitric acid oxidation of anibine.

(13) The loss of the methoxyl group in the alkaline cleavage presumably involves addition of hydroxide ion at the β-position followed by reverse Michael condensation with expulsion of methoxide. The initial product ($C_{10}H_7K_2NO_4 \cdot H_2O$), isolated in the alkaline cleavage of anibine, should then be represented by the hydrate of VI.

bine, the neutral product can only be 4-methoxy-6-piperonyl-α-pyrone (X).



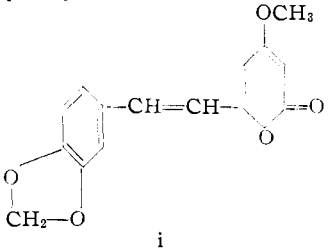
Aside from the steroidal toad poisons and squill glycosides,[14] α-pyrones are quite rare in nature.[15] It is interesting to note that the only two closely related naturally occurring α-pyrones, phenylcoumalin (6-phenyl-α-pyrone) (XI) and paracotoin (6-piperonyl-α-pyrone) (XII), are found in *Coto* and *Paracoto* bark.[16] Although it was known that the Bolivian *Coto* bark was derived from a tree belonging to the *Lauraceae*, all of the early medicinal and chemical work[17] was carried out when the taxonomic problem was still very confused.[18] Only the comparatively recent botanical revision of the *Lauraceae*[7] has resulted in the classification of *Coto* as *Aniba coto* (Rusby) Kostermans (syn. *Nectandra coto* Rusby) and of *Paracoto* as *Aniba pseudo-coto* (Rusby) Kostermans (syn. *Ocotea*[19] *pseudo-coto* Rusby). The striking chemical relationship between these four, naturally-occurring α-pyrones (IV, X, XI, XII) is thus paralleled by the close botanical connection of their plant sources, all of them belonging to the genus *Aniba*, and would suggest a common biogenetic origin. Synthetic experiments relating to these structures are now in progress.



(14) See C. Tamm in L. Zechmeister's "Progress in the Chemistry of Organic Natural Products," Vol. XIII, Springer, Vienna, 1956.

(15) See J. Fried in R. C. Elderfield's "Heterocyclic Compounds," Vol. I, John Wiley and Sons, New York, N. Y., 1950, pp. 354–370.

(16) Methysticin (i) and other 6-substituted-4-methoxy-5,6-dihydro-α-pyrones (*cf.* W. Borsche, C. H. Meyer and W. Peitzsch, *Ber.*, **60**, 2113 (1927)) isolated from *Piper methysticum* Forst. (fam. *Piperaceae*) are probably produced by quite a different biogenetic route from piperic acid and possibly acetate.



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(17) A complete review of the chemical and biological work is given by Messner, *Pharm. Zentr.*, **67**, 625, 680, 696 (1926).

(18) *Cf.* H. H. Rusby, *J. Amer. Pharm. Assoc.*, **11**, 775 (1922); C. W. Maplethorpe, *Pharm. J.*, **110**, 238 (1923).

(19) It should be noted that rosewood itself was once assumed to belong to this genus (see ref. 5).

Experimental[20]

Extraction Procedure.—The rosewood[21] was reduced to sawdust and then extracted exhaustively with benzene in a Soxhlet apparatus. After concentration, the basic fraction was removed with dilute hydrochloric acid and the latter made alkaline with ammonia. Crude anibine (IV) was filtered and additional quantities could be recovered by chloroform extraction to raise the yield to 2.6%.

The benzene solution was next extracted several times with 3% sodium hydroxide solution, washed with water until neutral, dried and evaporated to yield a solid residue mixed with essential oil from which it could be separated by direct filtration. The solid (*ca.* 2%) was best purified by chromatography in order to remove an orange-colored contaminant and afforded 1.2% of 4-methoxyparacotoin (X).

Anibine (IV).—Crystallization from 95% ethanol and sublimation at 130° and 0.003 mm. provided colorless crystals, m.p. 179–180°, which showed no perceptible rotation in methanol solution (*c* 0.1) over the range 700–400 mμ and did not give any color with alcoholic ferric chloride solution. The infrared bands (Nujol or chloroform solution) were found at 5.77, 6.02 and 6.33 μ (no OH or NH absorption) and are thus somewhat shifted from those reported for steroidal bufadienolides.[22] The ultraviolet spectral data are listed in Table I.

TABLE I

Solvent	Max. mμ (log ε)	Shoulder mμ (log ε)	Min. mμ (log ε)
EtOH	228.5(4.32), 315(4.09)	254(3.70)	269(3.67)
1 *N* HCl-EtOH	227.5(4.22), 322(3.95)	254(3.71)	278(3.42)
1 *N* KOH-EtOH (immediate)	253(3.84), 282(3.72), 381(4.16)		275(3.71), 300(3.65)
1 *N* KOH-EtOH (after 1 week)	334(3.36)		293(2.96)

Anal. Calcd. for $C_{11}H_9NO_3$: C, 65.02; H, 4.46; N, 6.89; O, 23.62; OCH_3, 15.27; mol. wt., 203. Found: C, 64.91; H, 4.56; N, 6.90; O, 23.31; OCH_3, 14.80; active H, 0.00; C-CH_3, 0.20; Rast mol. wt., 216.

The yellow **picrate** was prepared in ethanol solution and was recrystallized from the same solvent; m.p. 199–201°.

Anal. Calcd. for $C_{17}H_{12}N_4O_{10}$: C, 47.23; H, 2.80; N, 12.96. Found: C, 47.73; H, 2.50; N, 12.98.

Anibine hydrochloride was prepared by passing hydrogen chloride gas through an ethereal solution of anibine and recrystallizing from absolute ethanol. The decomposition point varied greatly m.p. 179–209° (dec. Kofler), 205–230° (dec. sealed capill.). As indicated in the Discussion, anibine can be removed in *ca.* 80% yield from aqueous hydrochloric acid by continuous extraction (14 hr.) with chloroform.

Anal. Calcd. for $C_{11}H_{10}ClNO_3$: C, 55.12; H, 4.21; Cl, 14.81; N, 5.84. Found: C, 54.87; H, 4.23; Cl, 14.34; N, 5.68.

The pale yellow **methiodide** was formed when a methanolic solution of anibine was left at room temperature overnight with an excess of methyl iodide. The precipitate was recrystallized once from methanol and dried *in vacuo* at room temperature; m.p. 233–236° dec.

Anal. Calcd. for $C_{12}H_{12}INO_3$: C, 41.75; H, 3.51; N, 4.06; O, 13.91; I, 36.77. Found: C, 41.88; H, 3.55; N, 3.95; O, 13.87; I, 36.85.

(20) All melting points were determined on the Kofler block. We are indebted to Mrs. Dolores Phillips for the spectral measurements and to Dr. A. Bernhardt (Mülheim, Germany) and Mr. Joseph F. Alicino (Metuchen, N. J.) for the microanalyses.

(21) The composition of both rosewood samples (*Aniba Duckei* and *A. rosaeodora*) in terms of their main constituents was qualitatively identical, but the yields recorded are for a sample of commercial *A. Duckei* from Manaus, Amazonas.

(22) R. N. Jones and F. Herling, *J. Org. Chem.*, **19**, 1252 (1954).

Acid Cleavage of Anibine (IV).—A solution of 200 mg. of anibine in 50 cc. of 5% hydrochloric acid was heated under reflux for 6 hr. in a stream of nitrogen, decarboxylation occurring during this process as evidenced by the precipitation of barium carbonate (88% yield) in a barium hydroxide trap. The solution was cooled, extracted continuously with chloroform for 17 hr., and the chloroform layer was washed with water, dried and evaporated. The residual semi-solid (55 mg.) was crystallized from petroleum ether and then sublimed at 80° and 0.005 mm.; m.p. 84–86°, red color with ferric chloride, $\lambda_{max}^{CHCl_3}$ broad band at 6.1–6.25 μ and small band at 5.80 μ. The ultraviolt absorption data are listed in Table II.

TABLE II

Solvent	Max., mμ (log ε)	Min., mμ (log ε)
EtOH	236(3.88), 309(4.15)	256(3.57)
1 *N* HCl–EtOH	251.5(3.83), 315(4.07)	236(3.73), 268(3.69)
1 *N* KOH–EtOH	245(3.79), 330(4.17)	275(3.47)

Identity with a synthetic specimen[11] of β-acetoacetylpyridine (III) was established by mixture melting point determination and infrared spectral comparison.

Anal. Calcd. for $C_9H_9NO_2$: C, 66.24; H, 5.56; N, 8.58; O, 19.61; C-CH_3, 9.20; active H, 0.61; mol. wt., 163. Found: C, 66.32; H, 5.42; N, 8.66; O, 20.22; OCH_3, 0.00; C-CH_3,[23] 9.42, 9.64; active H, 0.69; neut. equiv. (perchloric acid titration), 162.

Alkaline Cleavage of Anibine.—A solution of 200 mg. of anibine dissolved in 40–50 cc. of 1 *N* ethanolic potassium hydroxide was kept at 20° in an atmosphere of nitrogen. After 2–3 hr. crystals started to appear and these were filtered after 15 hr. and washed well with absolute ethanol; yield 163 mg., m.p. 240–250° dec. This virtually colorless potassium salt (VI) was insoluble in organic solvents, highly soluble in water and gave a red color with ferric chloride solution. The analysis as well as the infrared spectrum (λ_{max}^{Nujol} 3.03, 6.08, 6.18 and 6.29 μ) indicated the presence of water, but this could not be removed even on attempted azeotropic distillation with toluene.

Anal. Calcd. for $C_{10}H_7K_2NO_4 \cdot H_2O$: C, 39.85; H, 3.01; N, 4.65; K, 26.54. Found: C, 39.96; H, 2.91; N, 4.39; K, 25.79; OCH_3, 0.00.

The potassium salt VI could be transformed into the corresponding silver salt by dissolving in water and adding 5% aqueous silver nitrate solution. The pale yellowish precipitate was dried in a desiccator before analysis.

Anal. Calcd. for $C_{10}H_7Ag_2NO_4 \cdot H_2O$: C, 27.36; H, 2.07; N, 3.19; Ag, 49.15. Found: C, 26.94; H, 1.85; N, 2.97; Ag, 50.33.

A solution of 511 mg. of the potassium salt VI in 50 cc. of water was acidified with a few drops of hydrochloric acid and then extracted continuously with chloroform for 30 hr. The extraction was accompanied by decarboxylation as demonstrated by the formation of barium carbonate in a barium hydroxide trap attached to the extraction apparatus. The chloroform solution was dried, evaporated to dryness and the crystalline residue was sublimed at 80° and 0.005 mm. to yield 316 mg. of pure β-acetoacetylpyridine (III), m.p. 84–86°. This is the preferred procedure since the over-all yield based on anibine is 50%. The cleavage product readily forms a 2:1 adduct with silver nitrate in water solution.

Anal. Calcd. for $C_{18}H_{18}N_2O_4 \cdot AgNO_3$: C, 43.57; H, 3.66; N, 8.46; Ag, 21.74. Found: C, 43.35; H, 3.32; N, 8.72; Ag, 21.73.

Isolation of Nicotinic Acid from Cleavage of Anibine (IV).—In one experiment, the ethanolic potassium hydroxide solution of 150 mg. of anibine was left for only 1 hr., most of the ethanol was removed at room temperature *in vacuo* and water was added. Nothing could be extracted by means of chloroform and when the aqueous solution was neutralized with acetic acid, continuous chloroform extraction furnished only about 10 mg. of crude diketone III. The aqueous solution was then acidified with acetic acid and again extracted continuously with chloroform, whereupon 65 mg. of crude product was obtained. Sublimation at 120° and 0.005 mm. led to 15 mg. of nicotinic acid, m.p. 234–237°, undepressed upon admixture with an authentic specimen.

Anal. Calcd. for $C_6H_5NO_2$: C, 58.53; H, 4.09; N, 11.38; O, 25.99. Found: C, 58.81; H, 3.89; N, 11.40; O, 25.88.

Quantitative Microhydrogenations. (a) **Anibine (IV).**—A solution of 6.1 mg. of anibine in 5 cc. of glacial acetic acid was shaken with 7 mg. of platinum oxide at 25.5° with the following results (moles of H_2 (minutes)): 1 (5.5), 2 (14), 3 (25), 4 (39), 5 (52), 6 (65, 110). The sixth mole of hydrogen appears to be due to hydrogenolysis[10] which has already been observed earlier with α-pyrones.[24]

(b) **β-Acetoacetylpyridine (III).**—A solution of 6.9 mg. of the cleavage product III was treated under exactly the same conditions: 1 (4.5), 2 (10.5), 3 (17.5), 4 (29), 5 (78, 94).

4-Methoxyparacotoin (4-Methoxy-6-piperonyl-α-pyrone) (X).—The crude product was purified by chromatography on alumina deactivated with 3% of 10% acetic acid and eluted with benzene. Recrystallization from ethanol yielded the analytical sample as colorless needles, m.p. 222–224°, $[\alpha]D \pm 0°$ ($CHCl_3$); λ_{max}^{Nujol} 5.75, 6.04, 6.12 and 6.38 μ; λ_{max}^{EtOH} 336 mμ (log ε 3.98), λ_{min}^{EtOH} 272 mμ (log ε 3.44).

Anal. Calcd. for $C_{13}H_{10}O_5$: C, 63.41; H, 4.09; O, 32.49; OCH_3, 12.59. Found: C, 63.30; H, 4.07; O, 32.68; OCH_3, 12.73; C-CH_3, 0.00.

Alkaline Cleavage of 4-Methoxyparacotoin (X). (a) **With Boiling Alkali.**—A solution of 201 mg. of 4-methoxyparacotoin (X) was heated under reflux for 3 hr. with 20 cc. of 0.5 *N* ethanolic potassium hydroxide. After dilution with water, the neutral material was extracted continuously with chloroform yielding 93 mg. of cream colored solid, m.p. 53–80°.

Recrystallization from water gave 45 mg. of small, colorless needles of **3,4-methylenedioxyacetophenone (VII)**, m.p. 87–88°, $\lambda_{max}^{CHCl_3}$ 6.2 μ (broad).

Anal. Calcd. for $C_9H_8O_3$: C, 65.85; H, 4.91. Found: C, 66.35; H, 4.59.

An authentic sample was not available, but the constants of the ketone and its phenylhydrazone (m.p. 112–114°) were in good agreement with those reported in the literature.[25] Furthermore, sodium hypobromite oxidation furnished piperonylic acid.

The original aqueous solution was separated into phenolic (less than 10 mg.) and acidic fractions. The latter (34 mg.) represented a yellowish solid which crystallized from ethanol as colorless needles, m.p. 231–233°, undepressed upon admixture with an authentic sample of **piperonylic acid** (m.p. 231–233°).

(b) **At Room Temperature.**—A solution of 100 mg. of 4-methoxyparacotoin (X) was left at room temperature in 50 cc. of 1 *N* ethanolic potassium hydroxide solution for 24 hr. in a current nitrogen. The potassium salt (59 mg., λ_{max}^{Nujol} 3.15 and 6.30 μ) was filtered, dissolved in water and acidified. The colorless precipitate (30 mg.) of the **β-keto acid VIII** was filtered and dried at room temperature for analysis; m.p. 125–130° (gas evolution), λ_{max}^{Nujol} 5.82, 6.08 and 6.2 μ. It could not be recrystallized without effecting decarboxylation.

Anal. Calcd. for $C_{12}H_{10}O_6$: C, 57.60; H, 4.03; O, 38.37. Found: C, 58.23; H, 4.30; O, 37.12.

The β-keto acid VIII was either decarboxylated by first heating above its melting point, cooling and then subliming or by recrystallization from boiling water containing a small amount of ethanol. In each case, there was obtained colorless crystals of **1-piperonyl-butane-1,3-dione (IX)**, m.p. 91–

(23) On the other hand, Mr. Alcino reported that under the same conditions of the Kuhn–Roth oxidation, α-, β- and γ-picoline as well as 2,6-lutidine yielded no titratable acetic acid. Up to 90% of acetic acid could be obtained when the pyridine ring was activated (*e.g.*, 2-amino-4-methylpyridine, pyridoxine).

(24) See A. Stoll, A. Hofmann and H. Helfenstein, *Helv. Chim. Acta*, **18**, 644 (1935); J. Fried and R. C. Elderfield, *J. Org. Chem.*, **6**, 566 (1941).

(25) G. Ciamician and P. Silber, *Ber.*, **24**, 2989 (1891), report m.p. 88° for the ketone and m.p. 114° for its phenylhydrazone.

92°; $\lambda_{max}^{CHCl_3}$ 5.80 (small) and broad, strong band at 6.2 μ; λ_{max}^{EtOH} 238.5 and 335 mμ, log ϵ 4.02 and 4.29; λ_{max}^{EtOH} 253 mμ, log ϵ 3.64.

Anal. Calcd. for $C_{11}H_{10}O_4$: C, 64.07; H, 4.89; C-CH_3, 7.29. Found: C, 63.60; H, 4.82; C-CH_3, 8.13.[26]

(26) The high value may be due to small amounts of piperonylic acid carried over in the Kuhn-Roth determination.

The substance proved to be identical by mixture melting point determination and infrared comparison with a synthetic specimen (m.p. 92–93°) prepared by condensation of methyl piperonylate and acetone in ether solution in the presence of sodium.

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The Isoglutamine Isomer of Oxytocin: Its Synthesis and Comparison with Oxytocin[1]

BY CHARLOTTE RESSLER AND VINCENT DU VIGNEAUD

RECEIVED FEBRUARY 27, 1957

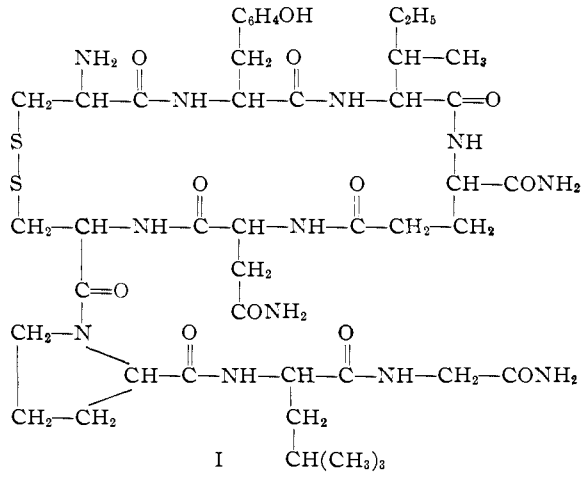
Synthesis of the isomer of oxytocin in which the glutamine residue has been substituted by isoglutamine is described. The change in the nature of the glutamic acid linkage with the consequent ring enlargement was found to result in virtually complete loss of the oxytocic and avian depressor activities of oxytocin. The *isoglutamine-oxytocin* differed from oxytocin also in some of its physical properties and exhibited a striking difference in optical rotation. However, several of the other properties of the two polypeptides were found to be indistinguishable.

The structure of oxytocin, the chief oxytocic principle of the posterior pituitary gland, has been established through its synthesis[2,3] as an octapeptide composed of a 20-membered cyclic disulfide ring branched by a tripeptide side chain, namely, the cyclic disulfide of L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-leucylglycinamide.

It may be recalled that this synthesis was based on structural studies[4] in this Laboratory on natural oxytocin and, in addition, on certain assumptions regarding the position of two amide groups and the nature of the linkages of the aspartic acid and glutamic acid residues. In proposing the structure for oxytocin[4,5] it seemed reasonable to assign the position of two amide groups to the aspartic acid and glutamic acid residues and, furthermore, it was assumed that these were present as asparaginyl and glutaminyl residues rather than as the isomeric isoasparaginyl and isoglutaminyl residues. After this structure for oxytocin had been established through synthesis, further confirmatory evidence with respect to the assumptions made was obtained by the demonstration of the presence of glutamine and asparagine in enzymatic hydrolysates of oxytocin and vasopressin.[6]

It was of considerable interest to determine whether oxytocin could be distinguished from the octapeptides isomeric with it with respect to the glutamine and/or asparagine residues. Such isomers would allow observations on the effect of a small structural change on the physical and chemical properties of oxytocin, and these results might be of interest with respect to the characterization of polypeptides and proteins. Comparison of the biological activities of oxytocin with those of its isoglutamine and/or isoasparagine isomer would provide, in addition, information bearing on the relationship of structure to the biological activities of the oxytocic hormone.

These considerations have led us to undertake the synthesis of the isoglutamine isomer of oxytocin. In this polypeptide an isoglutamine residue replaces the glutamine residue of oxytocin. It may be noted that the ring moiety of this isomer is larger by two methylene units than that present in oxytocin, and thus, *isoglutamine-oxytocin* (I) possesses a 22-membered ring.



I

The synthesis of isoglutamine-oxytocin was approached *via* the nonapeptide intermediate N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-isoglutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (VI), which corresponds in structure to the key intermediate used in the synthesis of oxytocin, with isoglutamine replacing the glutamine of the oxytocin intermediate. In the synthesis of oxytocin, suitable reduction of the nonapeptide intermediate followed by oxidation led to the desired 20-membered cyclic disulfide hormone. The projected synthesis of isoglutamine-oxytocin through a similar route, *i.e.*, by oxidation of the appropriate sulfhydryl nonapeptide, involved the as-

(1) This work was supported in part by a grant (H-1675) from the National Heart Institute, Public Health Service.

(2) V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, P. G. Katsoyannis and S. Gordon, THIS JOURNAL, **75**, 4879 (1953).

(3) V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts and P. G. Katsoyannis, *ibid.*, **76**, 3115 (1954).

(4) V. du Vigneaud, C. Ressler and S. Trippett, *J. Biol. Chem.*, **205**, 949 (1953).

(5) H. Tuppy, *Biochim. Biophys. Acta*, **11**, 449 (1953).

(6) H. C. Lawler, S. P. Taylor, A. M. Swan and V. du Vigneaud, *Proc. Soc. Exper. Biol. Med.*, **87**, 550 (1954).