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ERYTHROPHLEUM ALKALOIDS

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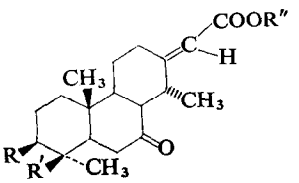
Abstract—Effective use of partition chromatography in the study of *Erythrophleum* alkaloids is reported. 8-Dehydrocassaic acid was isolated following hydrolysis of impure cassaine preparations, an indication of the existence of 6-hydroxycassaine. 8-Dehydrocassamine and several new derivatives of cassaine are described.

THE ERYTHROPHLEUM alkaloid cassaine (I) is usually isolated from bark extracts as its acid sulfate which is purified by multiple recrystallization of both the salt and the free base.[1]



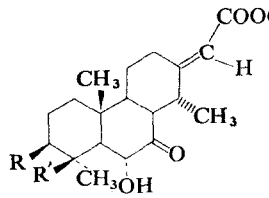
I R=OH, R′=CH_3,R″=$CH_2CH_2N(CH_3)_2$
II R=OH, R′=CH_3,R″=H
III R=OH, R′=CH_3,R″=CH_3
IV R=H, R′=$COOCH_3$,R″=$CH_2CH_2N(CH_3)_2$

Chromatography as described by Brown and Kupchan[2] has allowed much more efficient separation of cassaine from its accompanying alkaloids. The purest samples of the alkaloid were then obtained by recrystallization of the resulting free base. A hitherto unreported polymorphism was observed; thin plates and blades of m.p. 149–150° (evac. tube) separated from ether–pentane whereas heavy rectangular plates of m.p. 144–145·5° (evac. tube) crystallized from acetone.[3] The use of evacuated melting point tubes raised the m.p. 1–2°. The bisulfate salt prepared from this pure material showed no evidence of the previously reported hydration[1] even though it was recrystallized from the same system (H_2O–EtOH–ether).

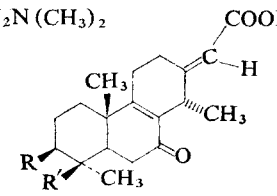
The mother liquors from the recrystallization of the free base as described above gave more crystalline material which showed a single spot corresponding to cassaine but with a lowered and broadened m.p. Hydrolysis of this material with dilute HCl produced the expected cassaic acid (II) along with 15–20 per cent of an impurity which ultimately proved to be 8-dehydrocassaic acid (VII), (isolated as its methyl ester (VIII)).

First indications of the identity of this impurity were provided by subtracting the u.v. absorption curve of the α,β-unsaturated ester chromophore of methyl cassaiate (III) from that of the unknown methyl ester to give a residual absorption at 248 nm (ϵ 9300), that of a highly substituted α,β-unsaturated ketone. The NMR spectrum showed only the

[1] G. DALMA, *Helv. Chim. Acta.* **22,** 1497 (1939).
[2] K. S. BROWN and S. M. KUPCHAN, *J. Chromatog.* **9,** 71 (1962).
[3] The generally accepted m.p. is that of Dalma's at 142·5° (ref. 1).

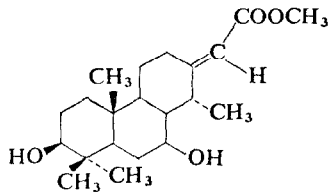


V R=H, R′=COOCH$_3$
VI R=OH, R′=CH$_3$

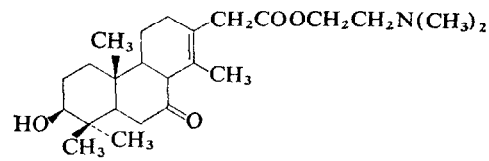


VII R=OH, R′=CH$_3$, R″=H
VIII R=OH, R′=CH$_3$, R″=CH$_3$
IX R=H, R′=COOCH$_3$, R″ = H
X R=H, R′=COOCH$_3$, R″=CH$_2$CH$_2$N(CH$_3$)$_2$

vinyl hydrogen of the unsaturated ester moiety. With the assumption that the unknown material was similar to cassaine, its methyl ester was tentatively identified as methyl 8-dehydrocassaiate (VIII). This structure was confirmed by reduction of VIII with $NaBH_4$ to methyl cassaidinate (XI)[4] which proved identical by direct comparison with an authentic ample prepared from cassaidinic acid.[4]



XI



XII

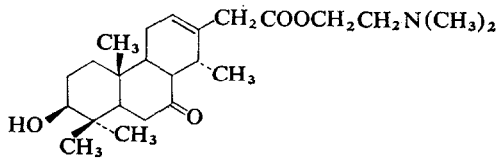
There are two major groups of *Erythrophleum* alkaloids. One group contains two methyl groups on C-4 and a hydroxyl on C-3 and is exemplified by cassaine (I). The other group, exemplified by cassamine (IV), has a β-carbomethoxy group at C-4. Recently Lindwall *et al.*[5] reported the isolation of erythrophleguine (V), which is 6α-hydroxycassamine. This compound undergoes dehydrative rearrangement to 8-dehydrocassamic acid (IX) in the course of hydrolysis with dilute HCl. Since the two series of *Erythrophleum* alkaloids are known to bear similar substituents, 6α-hydroxycassaine (VI) probably exists and would be the reasonable precursor of our isolated 8-dehydrocassaic acid (VII). Indeed, when the 'low melting cassaine' which furnished VII was checked by periodate titration, there was evidence of 12 per cent of α-hydroxy ketone, i.e. a strong indication that 6α-hydroxycassaine (VI) was present in our impure cassaine sample.

We, too, have isolated erythrophleguine (V)[5] from the alkaloid mixture, partition chromatography being particularly useful in this case. Its hydrolysis product, 8-dehydrocassamic acid (IX)[5] was converted *via* the acid chloride to 8-dehydrocassamine (X), a formal member of the *Erythrophleum* group which has not been isolated from natural sources.

There is considerable disagreement in the literature[6] over the existence of allocassaine (XII). It is purported to have a Δ^{13} bond as a result of migration from conjugation under

[4] L. RUZICKA and G. DALMA, *Helv. Chim. Acta* **23**, 753 (1940).
[5] (a) O. LINDWALL, F. SANDBERG, R. THORSÉN and T. NORIN, *Tetrahedron Letters* 4203 (1965); (b) O. LINDWALL, T. NORIN, F. SANDBERG and R. THORSÉN, *Acta Pharm. Suecica* **2**, 313 (1965); (c) A. THORELL, S. AGURELL, F. SANDBERG and T. NORIN, *Acta Chem. Scand.* **22**, 2835 (1968).
[6] Cf. F. FALTIS and L. HOLZINGER, *Chem. Ber.* **72**, 1443 (1939).

alkaline conditions. Pyrolysis of cassaine at 250° *in vacuo* caused at least some bond migration in the opposite direction. A mixture is formed from which the Δ^{12} compound XIII, designated *pyrocassaine*, can be isolated.



XIII

In the course of our work we prepared and report here three incidental compounds, cassaic acid 3-acetate (I, R=CH_3COO, R′=CH_3, R″=H), cassaine 3-ethyl carbonate (I, R=EtOCOO, R′=CH_3, R″=$CH_2CH_2N(CH_3)_2$), and cassaine methiodide. This methiodide is said to form[6] but experimental conditions and properties of the compound have not been reported.

EXPERIMENTAL[7]

Isolation of Cassaine from Erythrophleum guineense *Bark*

An Et_2O solution of crude alkaloid mixture, separated from 50 kg of *Erythrophleum guineense* bark[8] according to the method of Dalma,[1] was treated with ethereal H_2SO_4 which caused precipitation of a gum. A suspension of this crude sulfate in 300 ml of H_2O was made strongly basic with conc. NH_4OH and the precipitated base was extracted with 2 l. of Et_2O. Concentration of the extracts gave 34 g of crude cassaine base which was purified by partition chromatography in three portions using the method of Kupchan.[2] The biphasic solvent system used was a mixture of 0·2:2:1:8 H_2O–MeOH–$(CH_2Cl)_2$–hexane. A column 9 cm in diameter was packed with 300 g of Supercel which had been wet with 225 ml of polar phase containing 75 mg of bromocresol purple and brought to a faintly yellow color by means of gaseous HCl. The sample (12 g) was dissolved in 24 ml of $CHCl_3$ and dispersed on 24 g of Supercel. The $CHCl_3$ was allowed to evaporate and the dry powder was placed on the top of the column. Elution with the non-polar phase moved the leading edge of the main band (purple) to within 4 cm of the bottom of the column with a light purple color extending ahead to the bottom. A clearly-defined band trailed the main band.

The bands were scraped from the column and eluted with $(CH_2Cl)_2$ which simultaneously eluted the indicator. The eluate was concentrated to a 100-ml volume and 400 ml of hexane was added to precipitate the indicator selectively. Filtration and concentration of the filtrate then afforded the product.

The bottom 4 cm of the three columns gave a total of 7 g of solid which proved to be nearly pure cassaine. It ran ahead of the main band, presumably owing to overloading of the column. Upon rechromatography it ran normally like the main cassaine band, affording 6·5 g of this alkaloid. When dissolved in 25 ml of acetone and precipitated with 25 ml of Et_2O and 100 ml of pentane, this cassaine crystallized in long, thin plates and blades (4·65 g) of the high-melting polymorph, m.p. 145–148°. Further recrystallization of such material by concentration of an Et_2O solution followed by addition of pentane gave plates and blades, m.p. 149–150° (evac. tube); $[\alpha]_D^{25}$ − 105° (1% in EtOH); u.v.$_{max}$ 224 nm (ϵ 18,000). In an open tube, this sample melted at 148–149°. (Found: C, 70·9; H, 9·7; N, 3·5. $C_{24}H_{39}NO_4$ required: C, 71·06; H, 9·69; N, 3·45%.)

The main cassaine bands from the partition columns furnished a crystalline solid which was dissolved in 60 ml of acetone. Addition of 60 ml of Et_2O and 200 ml of pentane precipitated 7·72 g of cassaine, m.p. 138–143°. When this solid was dissolved in 200 ml of acetone in an open flask and the solvent was allowed to evaporate slowly, thick, rectangular plates of a low melting polymorph separated, 2·85 g, m.p. 144–145·5°. The m.p. of further crops dropped sharply. The total cassaine of satisfactory purity isolated was thus 10·2 g.

Rechromatography (partition) of all mother liquor residues and recrystallization failed to give further pure cassaine even though the partition column showed a single band and TLC on a silica plate developed with *i*-$PrNH_2$–MeOH–$CHCl_3$ (3:3:94) showed a single spot. The solid melted at 120–140°. It (11·9 g) was the source of the *methyl 8-dehydrocassaiate* (VIII) described below.

A 2·0-g sample of cassaine in 5 ml of $(CH_2Cl)_2$ was diluted with 25 ml of Et_2O and treated with ethereal

[7] All m.ps are corrected.

[8] Obtained from S. B. Penick Co. with only the information that its origin was the Belgian Congo. Dalma[1] indicates that the alkaloid content of this bark varies considerably with locality.

H_2SO_4 until precipitation was complete. The resulting 2·8 g of solid was dissolved in 80 ml of H_2O at 25° and the solution was diluted with 210 ml of absolute EtOH followed by 1·3 l. of Et_2O. Thin plates of *anhydrous cassaine* bisulfate separated which were collected and dried at 55° (2 mm) for 3 hr. This salt (1·8 g) melted at 305–306° dec.; $[\alpha]_D^{25}$ − 96° (1% in H_2O). (Found. C, 57·23; H, 8·21; S, 6·37.$C_{24}H_{39}NO_4 \cdot H_2SO_4$ required: C, 57·23; H, 8·21; S, 6·37%.) The crystals showed no evidence of cracking or opaqueness which usually accompanies loss of water crystallization.

Isolation of Methyl 8-Dehydrocassaiate (VIII)

An 11·8-g sample of mother liquor residue of m.p. 120–140° described above was dissolved in 200 ml of hot 2N HCl and the solution was heated at 100° for 6 hr. The mixture was cooled and the precipitated solid was collected and air-dried (8·95 g). This product showed two spots on a silica chromatoplate developed with HOAc–CH_2Cl_2–pentane–Et_2O. (2:20:28:50).

Chromatography of 10·8 g of such product on 400 g of silica gel using HOAc–CH_2Cl_2–Et_2O–pentane (1:20:20:59) gave 1·40 g of pure *cassaic acid* (II) followed by mixtures of it with *8-dehydrocassaic acid* (VII). The cassaic acid trailed so badly throughout the second component that purification of the latter by this method was not feasible.

The 5·9 g of acid mixture from the column was dissolved in 100 ml of MeOH and the solution was treated with ethereal CH_2N_2 prepared from 15·4 g of 'Diazald'.[9] After 16 hr the solution was concentrated to a residue by warming *in vacuo* and the residue (in 20 ml CH_2Cl_2 and 200 ml Et_2O) was washed with 20 ml of 1N NaOH and brine and concentrated to a residue which solidified.

Chromatography of this residue on 400 g of silica gel using Et_2O–pentane (35:65 → 50:50) gave 1·00 g of pure (single spot) methyl cassaiate (III) followed by 2·89 g of a mixture and then by 0·53 g of pure (single spot) methyl 8-dehydrocassaiate (VIII). The latter compound was recrystallized from ether with pentane added to give 0·23 g of colourless blade clusters, m.p. 144–146°. The mother liquor was added to the mixture above for rechromatography.

The process of chromatography was repeated four more times with separation of some pure lead and trail components. Finally, the remaining 0·76 g of mixture was separated on fifteen 20 × 40-cm thick-layer silica plates using Et_2O for development. In this manner a total of 3·0 g of methyl cassaiate (III) and 0·99 g of methyl 8-dehydrocassaiate (VIII) were obtained.

Recrystallization of the methyl 8-dehydrocassaiate from ether gave colorless blade clusters, m.p. 145–146·0°; $[\alpha]_D^{25}$ − 80·5° (1% in $CHCl_3$); u.v.$_{max}$ 224 nm (ϵ 18,900), sh at 250 nm; i.r. (KBr) 2·89 (OH), 5·82 ($COOCH_3$) and 5·99 μ (conj. C=O), NMR δ 0·90, 1·03, 1·08 (s, three CH_3 at C-4 and C-10), 1·08 and 1·20 (d of C-14 CH_3), 3·70 (OCH_3 of ester), 5·81 ppm (=CH). (Found: C, 71·7; H, 8·9. $C_{20}H_{30}O_4$ required: C, 71·82; H, 9·04%.)

Reduction of Methyl 8-Dehydrocassaiate (VIII)

A solution of 67 mg of methyl 8-dehydrocassaiate (VIII) in 4 ml of 94% EtOH was treated with an equal weight of $NaBH_4$ and the solution was allowed to stand for 18 hr. Addition of 3 ml of 2N HCl and concentration of the solution by warming *in vacuo* gave a residue which was partitioned between water and ether. The ether layer was separated, washed with brine, dried (Na_2SO_4) and concentrated to a residual oil.

Chromatography of this oil on 3 silica-coated, 20 × 40-cm plates developed with ether revealed two prominent bands which account for perhaps 90% of the mixture. The less-polar, oily material (25 mg) was not investigated. The more-polar material was rechromatographed to give crystalline *methyl cassaidinate* (XI), m.p. 159·5–165·5°. Recrystallization from ether with hexane added gave 20 mg of spherulites which appeared to start to melt about 140°, resolidified and then melted at 165·5–167° (polymorphism). The authentic (XI)[4] prepared in the following experiment, showed the same polymorphic behavior and a mixture m.p. of the two materials showed no depression. The i.r. spectral curves of the samples corresponded in all 23 peaks but a slight purity difference, reflected in the difference in m.p., caused slight differences in band intensities. This purity factor was also evident in the mass spectral curves which were essentially identical.

Methyl Cassaidinate (XI)[4]

This compound was prepared from cassaidinic acid by reaction with CH_2N_2 as reported by Ruzicka and Dalma[4] with the following modified workup and notation on polymorphism. The crude product was chromatographed on one 20 × 40-cm silica coated plate with development by Et_2O. The sole band visible under u.v. light was eluted with Et_2O to give a solid melting at 160–161·5°. When this solid was dissolved in ether, the solution concentrated to a 3-ml volume and cyclohexane added to produce faint cloudiness, tight needle clusters separated which slowly changed to rather transparent spherulites. This solid (47 mg) melted at 162·5–164° when inserted at 135° but melted immediately when inserted at 143°. The melting point of this low-melting form appears to be 138°. Ruzicka and Dalma[4] report 162–163°.

[9] Aldrich Chemical Co., Milwaukee, Wisconsin.

Isolation of Erythrophleguine (6α-Hydroxycassamine) (V)

The mother liquor residues from the crystallization of the crude alkaloid sulfates, called *Mother Liquor A* in the bark extraction procedure above, were converted to the free base form and chromatographed according to the Kupchan procedure[2] using H_2O–MeOH–$(CH_2Cl)_2$–hexane (0·2:2:1:12). Some yellow, oily material was eluted first and just ahead of the principal basic fraction which contained erythrophleguine.[6] Cassaine was eluted only very slowly with this system. The process was repeated to effect further purification and then the erythrophleguine was finally purified as its bisulfate salt, m.p. 169–171·6° (intumescence) (needle clusters from MeOH with ether added); $[\alpha]_D^{25}$ − 30·4° (1% in EtOH); u.v.$_{max}$ 224 nm (ε 19,000).

8-*Dehydrocassamine (X)*

A solution of 1·10 g (3·0 m-moles) 8-dehydrocassamic acid (IX) in 10 ml of MeOH and 30·6 ml of 0·1 N NaOH (3·0 m-moles) was concentrated to a residue at <40° *in vacuo*. This residue was suspended in 30 ml of C_6H_6 and the C_6H_6 was removed at <40° *in vacuo* in order to remove residual H_2O. The drying process was repeated with 30 ml C_6H_6–EtOH (1:1) and with 30 ml of C_6H_6. The residue was finally heated at 100° *in vacuo* for 2 min. This sodium salt was suspended in 25 ml of dry C_6H_6 at room temperature and treated with 5 ml of oxalyl chloride. No significant heat was evolved. After 12 min the excess oxalyl chloride was removed *in vacuo* and 5 ml of dry C_6H_6 was added followed by 2·0 ml of 2-dimethylaminoethanol. This mixture was boiled for 3 min and then concentrated to a residue. The residue was dissolved in H_2O and Et_2O, 10 ml of 2N NH_4OH was added and the layers were separated. The Et_2O layer was washed once with water and then extracted twice with 1N HCl. The extracts were made alkaline with 2N NH_4OH and the liberated base was separated with ether. This solution was dried (Na_2SO_4) and concentrated to give 1·08 g of colorless oil.

Partition chromatography of the crude basic ester on 100 g of Supercel as described in the purification of cassaine above showed only one basic material in significant quantity. Elution of this band afforded 1·04 g of oil which was dissolved in 25 ml of Et_2O and treated with gaseous HCl. The crystalline HCl salt (1·11 g, single tlc spot on silica plate with *i*-$PrNH_2$–MeOH–$CHCl_3$, (1:1:48) R_f 0·5) in 3 ml of hot MeCN was diluted with 3 ml of Et_2O to give tight needle clusters (0·92 g, 66%) of (X), m.p. 195–197°. One further recrystallization raised this m.p. to 196·5–198·5; $[\alpha]_D^{25}$ + 56·0° (1% in $CHCl_3$); u.v.$_{max}$ 229 nm (ε 20,100); i.r. (KBr) 5·80, 5·82 (sh), 6·00 (3 carbonyl groups) and 6·13 μ (exo C=C); NMR (ext. TMS) δ 6·30 (s, 1, =CH), 4·15 (s, 3, $COOCH_3$), 3·45 (s, 6, NMe_2), 1·72 and 1·60 (unequal d, 6, $-\overset{|}{C}-CH_3$ and C-14 CH_3), and 1·45 ppm (s, 3, $-\overset{|}{C}-CH_3$). (Found: C, 64·4; H, 8·3; Cl, 7·8. $C_{25}H_{37}NO_5 \cdot HCl$ required: C, 64·28; H, 8·20; Cl, 7·59%.)

Substraction of the α,β-unsaturated ester chromophore of cassaine from that of this product results in a very symmetrical u.v. peak at 247 nm (ε 11,400).

Pyrocassaine (XIII)

A 3·00-g sample of cassaine was placed in a flash which was flushed with N_2 and then evacuated to a pressure of 0·07 mm. When the flash was heated by a Wood's metal bath at 250° the alkaloid melted with vigorous bubbling and the resulting oil refluxed in the flask. Heating was continued for 1 hr. The oil was dissolved in Et_2O containing about 10% CH_2Cl_2 and washed with two 5-ml portions of 2N HCl, 5 ml H_2O, 10 ml 2N NaOH and finally with H_2O. The Et_2O solution yielded 0·84 g of neutral oil which contained four products in a 4:2:1:1 ratio (by silica TLC plate developed with 3:3:94 MeOH–*i*-$PrNH_2$–THF). This material was inadvertently lost.

Acidification of the basic extract yielded 0·17 g of solid which was not investigated.

Basification of the HCl extract afforded 1·68 g of oil which was purified on a 350-g partition column as described above in the purification of cassaine. The principal band afforded 1·31 g of oily XIII which was converted to a solid HCl salt. Recrystallization of this salt from CH_3CN (Et_2O added) and then from CH_3CN furnished 0·55 g of needles (or plates which reverted to needles, i.e. polymorphism), m.p. 200–202°. Further recrystallization from CH_3CN gave needles, m.p. 211–212·5° (evac. cap), $[\alpha]_D^{25}$ + 60·4 (1% in H_2O), u.v. shows slight shoulder at 218 nm on end absorption, NMR δ 6·00 (s, 1, vinyl H), 3·62 (s, $=\overset{|}{C}-\underset{O}{\overset{||}{C}}H_2C$). (Found: C, 64·9; H, 9·2; Cl, 8·5. $C_{24}H_{39}NO_4 \cdot HCl$ required: C, 65·21; H, 9·12; Cl, 8·02%.)

Cassaic Acid 3-Acetate (I, R=CH_3COO,R'=CH_3,R''=H)

Crude cassaic acid (3·6 g) was dissolved in 12 ml of Ac_2O and 24 ml pyridine and the solution was heated on the steam bath for 1·5 hr. Excess reagents were removed by warming *in vacuo* and the residual oil was dissolved in Et_2O. This solution was shaken for 5 min with 60 ml of 2N HCl, washed with H_2O and dried over Na_2SO_4. Concentration of the solution gave a residual oil which was chromatographed on 225 g of silica gel using AcOH–Et_2O–pentane (1:15:84). The desired 3-acetate was eluted first, affording 0·75 g of

crystalline solid. Recrystallization by dissolving the solid in 3 ml of THF and diluting the solution with 16 ml of hexane gave 0·55 g of needle clusters (or massive prisms of metastable polymorph) which melted at 206·5–209·5°; m.p. unchanged by further recrystallization; $[\alpha]_D^{25}$–83·8° (1% in $CHCl_3$); u.v.$_{max}$ 219 nm (ϵ 15,100); NMR spectrum compatible with structure; single spot on silica chromatoplate developed with 2:58:40 AcOH–Et_2O–pentane. (Found: C, 70·4; H, 8·4; Neut. Equiv., 378. $C_{22}H_{32}O_5$ required: C, 70·18; H, 8·57; Neut. Equiv., 376·5.)

Cassaine Ethyl Carbonate (I, R=EtOCOO,R'=CH_3,R''=$CH_2CH_2N(CH_3)_2$)

A solution of 0·8 g of cassaine in 7 ml of pyridine was treated with 1·5 ml of ethyl chloroformate with stirring at 15–20° in 3 min. At this point heavy precipitation of a solid caused the mixture to set to a cake which then became homogeneous again by the end of a 30-min period. The solution was allowed to stand at room temp. for 16 hr and concentrated to a residue at < 50° *in vacuo*.

The residue was dissolved in 50 ml of H_2O, the solution was made strongly basic with NH_4OH and the precipitated product was extracted by two portions of Et_2O. This crude product was purified by partition chromatography on 60 g of Supercel as described above for the purification of cassaine. The leading (and largest) band on the column was eluted with non-polar solvent phase to give 0·60 g of colorless solid which was recrystallized from 10 ml of hexane with concentration to a 1-ml volume. Diamond-shaped plates (0·52 g) separated upon cooling, m.p. 105–109·5°. Two further recrystallizations furnished 0·41 g of cassaine ethyl carbonate, m.p. 110–111°, $[\alpha]_D^{25}$–73° (1% in EtOH). (Found: C, 67·8; H, 9·3. $C_{27}H_{43}NO_6$ required: C, 67·90; H, 9·07%.)

Cassaine Methiodide

A solution of 1·37 g of cassaine in 20 ml of $CHCl_3$ was treated with 5 ml of CH_3I at room temperature. An oil began to precipitate within 5 min; it slowly solidified. The solid was collected, washed with ether and recrystallized from 30 ml of CH_3CN to give 0·68 g of colorless needles, m.p. 260–261° dec. Concentration of the filtrate to a 10-ml volume and cooling afforded 0·57 g more of the methiodide with the same m.p. (1·25 g, 68%); $[\alpha]_D^{25}$ – 88·8° (1% in H_2O). (Found: C, 54·5; H, 8·0; I, 22·8. $C_{25}H_{42}INO_4$ required: C, 54·84; H, 7·73; I, 23·18%.)